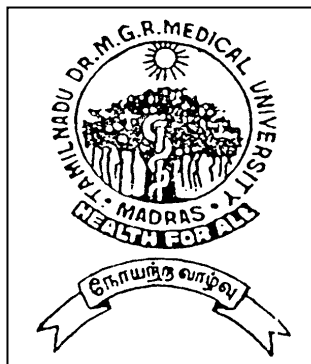


**A HISTOPATHOLOGICAL STUDY OF
MALIGNANT ROUND BLUE CELL TUMORS
WITH IMMUNOHISTOCHEMICAL EVALUATION**

**DISSERTATION SUBMITTED FOR
M.D BRANCH III
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CERTIFICATE

This is to certify that the dissertation entitled “**A Histopathological study of Malignant Round Blue Cell Tumors with Immunohistochemical Evaluation**” submitted by **Dr. K.Viswanathan** to the Faculty of Pathology, The Tamilnadu Dr. M.G.R. Medical university, Chennai in partial fulfilment of the requirement for the award of M.D. Degree in Pathology is a bonafide work carried out by him during the period June 2008 - May 2010 under my direct supervision and guidance.

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INTRODUCTION

Malignant Round Blue Cell Tumors comprise a diverse group of diagnostically challenging primitive (or) undifferentiated neoplasms which composed of small cells with uniform round nuclei and scanty cytoplasm have been referred as small-cell, round-cell, or blue cell tumors. “Round” refers to uniform shape of the nuclei, “blue” to colour of the nuclei on routine hematoxylin and eosin staining and “small” to the relative lack of cytoplasm. The “small cells” of small cell tumors are larger than most inflammatory cells⁸⁴. The tumors included in this category of neoplasms are as follows^{13, 49,74,81,83,}

1. Non Hodgkin’s Lymphoma
2. Ewing’s sarcoma /Primitive neuroectodermal tumor (PNET)
3. Embryonal and Alveolar Rhabdomyosarcoma
4. Wilm’s tumor
5. Neuroblastoma
6. Desmoplastic Small Round Cell Tumor (DSRCT)
7. Mesenchymal Chondrosarcoma
8. Small cell Osteosarcoma
9. Small cell carcinoma
10. Malignant Rhabdoid tumor
11. Myxoid Chondrosarcoma

12. Poorly differentiated synovial sarcoma
13. Round cell Liposarcoma
14. Epithelioid sarcoma with rhabdoid phenotype
15. Retinoblastoma
16. Medulloblastoma

Immunohistochemistry (IHC) and molecular diagnostics have greatly improved our ability to differentiate the various tumors and classify some of these tumors^{10, 81}. Use of the special diagnostic techniques have become increasingly important in diagnosis, evaluation of recurrent or metastatic disease, and in some cases for prognostic classification. These are always to be interpreted after careful evaluation of the light microscopic findings. However, the more we use these tools diagnostically, the more it becomes apparent that these special techniques have pitfalls of their own^{43,56}. Finally, additional techniques such as flow cytometry, electron microscopy and molecular analysis may be required for final confirmation of the diagnosis (or) to provide prognostic information^{52, 53}.

AIM OF THE STUDY

1. To find out the incidence of Malignant Round Blue Cell Tumors in and around Madurai.
2. To know the age and sex incidence of Malignant Round Blue Cell Tumors.
3. To correlate the clinical and histopathological features of Malignant Round Blue Cell Tumors.
4. To study the role of special stains in selected cases.
5. To confirm the histopathological diagnosis by Immunohistochemistry wherever necessary.
6. To classify the Malignant Round Blue Cell Tumors by Immunohistochemistry
7. To aid the clinicians for the management of the patients.

REVIEW OF LITERATURE

Nomenclature

Neoplasia means new growth. The term 'Tumor' is originally applied to the swelling caused by inflammation, but the non neoplastic usage of tumor has almost vanished. The term tumor is now equated with neoplasm. Oncology (Greek oncos=tumor) is the study of tumors or neoplasm.

The eminent British Oncologist Willis has come closest: "A neoplasm is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after cessation of the stimuli which evoked the change"⁸³.

Neoplasms are generally divided into

1. Benign
2. Malignant

Benign tumors

In which both microscopic and gross characteristics are considered relatively innocent. It will remain localized. It cannot spread to other sites, and it is generally amenable to surgical removal.

Malignant tumors

These are collectively referred to as cancers, derived from Latin word for ‘crab’, because they adhere to any part that they seize on in an obstinate manner. Malignant tumors invade and destroy adjacent structures and spread to distant sites (metastasize) to cause death⁸³.

Definition of small round cell tumors

“Denny” defined the characteristics of round blue cell tumors. Malignant Round Blue Cell Tumors can be defined in terms of clinical and pathological features i.e. the routine histological, immunohistochemical and ultrastructural features and by genetic markers. Although microarray analyses can discriminate between types of these tumors, a competent pathologist can do it with equal accuracy by using routine histological and ancillary morphologic techniques¹⁸.

Malignant Round Blue Cell Tumors – Table -1

Basic conventional spectrum

1. Neuroblastoma (NB)
2. Ewing’s sarcoma / peripheral (primitive) neuroectodermal tumor (ES/PNET)
3. Rhabdomyosarcoma (RMS)
4. Non Hodgkin’s lymphoma (NHL)

Extended spectrum

1. Desmoplastic small round cell tumor (DSRCT)
2. Small cell undifferentiated Osteosarcoma (UOS)
3. Small cell Hepatoblastoma
4. Wilm's tumor (WT)
5. Small cell variant of synovial peripheral nerve sheath tumors (MPNST)
6. Small cell variant of synovial sarcoma (SS)
7. Undifferentiated Small Round cell Tumor (SRCT).

Accurate diagnosis of the tumor type is necessary for selection of appropriate treatment protocol⁷³. The clinical scenario, primary site, metastatic pattern, gross and microscopic findings are useful to conclude the diagnosis whereas other features such as immunohistochemistry, electron microscopy and genetic features provide definitive criteria for the diagnosis^{26,27}. Treatment modality varies with each tumor type, so accurate diagnosis of the tumor type is mandatory. For example, a therapeutic protocol for Ewing's sarcoma is chemotherapy and local radiotherapy whereas surgery is not mandatory. The Neuroblastoma can be cured by surgical excision^{35,48}.

Table-2

Classification of small round cell tumors⁶²

Traditional	Revised
1. Ewing's sarcoma	1. pPNET (Bone and soft tissue)
2. Neuroblastoma	a. Ewing's sarcoma
3. Rhabdomyosarcoma	b. Peripheral neuroepithelioma
4. Lymphoma	c. 'Askin' tumor of chest wall
	d. PNET of bone
	e. Extra osseous Ewing's (Neural)
	2. Round cell bone tumors
	a. Small cell Osteosarcoma
	b. Mesenchymal Chondrosarcoma
	c. Primitive sarcoma of bone
	3. Round cell soft tissue sarcoma
	a. Poorly differentiated Rhabdomyosarcoma
	b. Extra osseous Ewing's (non neural)
	4. Metastatic Neuroblastoma
	5. Extra nodal lymphoma

ANALYSIS OF INDIVIDUAL TUMOR CHARACTERISTIC

HISTORICAL PERSPECTIVE

Non Hodgkin's Lymphoma

In 1858 Rudolph Virchow, a great German pathologist described lymphoma as a primary malignant neoplasm of the lymphoid tissue, which includes Hodgkin's lymphoma (HL) and Non Hodgkin's lymphoma (NHL)¹¹. In 1863, he described lymphoma as malignant tumor derived from lymphoid cells known as "Lymphosarcoma".

In 1871, Billroth used the term "Malignant lymphoma". In 1893 Dreshfeld and Kundrat made the first attempt to delineate subcategories of lymphoma. In 1908, Sternberg described an aggressive mediastinal tumor in young males, initially known as "Sternberg sarcoma" and later recognized as Lymphoblastic Lymphoma. In 1913 J.Ewings suggested that the tumor was derived from reticulum cells..In 1923 Evald introduced the term 'Reticuloendotheliosis' to represent hyperplasia of reticuloendothelial tissue¹¹. In 1928, C.Oberling introduced the term 'Reticulosarcoma' for neoplasm derived from lymphoreticular system.

Ewing's sarcoma / PNET

In 1921, James Ewing reported a tumor in radius which is composed of round cells and calling it as a “Diffuse endothelioma of bone” and proposing an endothelial derivation⁷⁸.

In 1928, OBERLING introduced the term Ewing's sarcoma, which was a non-committal name to the cell of origin. Seemayer et al described peripheral neuroectodermal tumors (PNETs) which were unrelated to peripheral (or) sympathetic nervous system⁷⁸.

In 1979 Askin et al described the “malignant small cell tumor of the thoracopulmonary region” (Askin tumor) having histological features similar to that of PNETs^{32, 47}.

Identification of common cytogenetic abnormality t (11;22) q (24;12) in Ewing's sarcoma and PNET favours the hypothesis that these two are histogenetically related. Ewing's sarcoma and PNET collectively called as “Ewing Family of Tumors”, form a single neoplastic entity sharing common histological and molecular features, and differing only in their extent of cellular differentiation^{5, 16}.

Rhabdomyosarcoma (RMS)

RMS was first described by Webber¹⁹. Masson referred the RMS as “Rhabdopoietic sarcomas”. RMS is a malignant tumor of mesenchymal origin.

RMS is thought to arise from immature mesenchymal cells that are committed to skeletal muscle lineage. But these tumors can arise in tissues in which striated muscle is not normally found.eg. Urinary bladder¹⁴.

Wilm's tumor

In 1814 Wilm's tumor was reported by Rance. In 1899 the condition was named after Carl Max Wilhelm Wilms, a German doctor who was one of the person, first to study this disease. In 1964 Miller and Colleague reported an association between aniridia and Wilm's tumor. Subsequently, a rare complex of developmental anomalies including aniridia, genitourinary malformation and mental retardation were identified and known as WAGR syndrome⁷.

The synonyms used for Wilm's tumor includes nephroblastoma, embryoma, carcinosarcoma, adenosarcoma and adenomyosarcoma³⁸.

Neuroblastoma

In 1864 Virchow first described its histological appearance and published an article entitled "Hyperplasia of the pineal and suprarenal glands" and he noted nodular distension of the suprarenal gland with three or more pea or cherry sized swelling which arose from the adrenal medulla²³.

Virchow described large numbers of sympathetic cells apart from the usual elements of adrenal medulla and he classified these tumors with gliomas at that time²³.

In 1901, W.Pepper reported a study of “congenital sarcoma of the liver and suprarenal gland”. These were probably the first patients with the disease type, now described as stage 4s Neuroblastoma²³.

In 1910 J.H.Wright first used the term Neuroblastoma. He noted that the migration of primitive nerve cells during embryogenesis explained the development of tumors of similar appearance in various sites of the body²³.

J.B.Beckwith and R.F.Martin defined a method of grading of tumors from 1 to 4 depending on the degree of differentiation, and equated lymphocyte infiltration of the neoplasm with a better prognosis. H.Shimada et al developed a grading system based on mitosis, karyorrehexis index, amount of stroma and the patient’s age²³.

Mesenchymal Chondrosarcoma

This rare neoplasm was first described by Lichtenstein and Bernstein in 1959 as an unusual variant of Chondrosarcoma which usually affects bone and soft tissue^{49,84}.

Small cell Osteosarcoma

This tumor is a distinctive microscopic variant of Osteosarcoma, described in 1979, as a tumor simulating Ewing’s sarcoma. They appear to represent a definite histological entity and the prognosis may be worse for these lesions than conventional Osteosarcoma.

It is most important not to misdiagnose this tumor as Ewing's sarcoma because it appears not to be radiosensitive. Radical surgery and modern adjuvant chemotherapy seem to offer the best chance of cure^{22,49}.

Incidence of Malignant Round Blue Cell Tumors

In India lymphoma is the second most common childhood tumor after acute leukemia, but in developed countries central nervous (CNS) tumors are the second most common and lymphomas are in third place. In India the overall incidence of Non Hodgkin's Lymphoma in males is 3.2% and 1.7% in females⁸².

Ewing's sarcoma / PNET constitute around 6-10% of primary malignant bone tumors and is the second most common primary malignant bone tumor. Extra skeletal Ewing's is the second most common pediatric soft tissue sarcoma^{61,71}.

Rhabdomyosarcoma is the most common pediatric soft tissue sarcoma composed of 80-90% of reported cases^{74,76}.

Wilm's tumor affects 1 per 10,000 children worldwide before the age of 15, and ranks fifth in incidence among the solid tumors of the childhood, following central nervous tumor, lymphoma, Neuroblastoma and soft tissue sarcoma^{31,55,70}.

Neuroblastoma is the most common extra cranial solid tumor in children, and the most frequently diagnosed tumor of infancy. It occurs at a rate of 1 per 10000 live births. It accounts for 7-8% of all paediatric malignant tumors^{70,79}.

Age and sex

In children Non Hodgkin's lymphoma is less common than Hodgkin's lymphoma but it is reverse in adults⁸². Surveillance Epidemiology and End Results (National Cancer Institute.USA) showed 67 years as the median age at diagnosis of NHL. Non Hodgkin's Lymphoma predominantly affects males with a male: female ratio of 1.4:1⁸⁰.

Wilkins et al states that Ewing's sarcoma occurs between the age group of 5 to 20 years with a distinct male preponderance⁸⁶. Carvajal R et al found that common age group for Ewing's sarcoma / PNET is 10-20 years, with a slight male predominance⁶.

Embryonal type of Rhabdomyosarcoma (RMS) affects children commonly below 15 yrs. Alveolar RMS affects mainly those between the ages of 10-25 years⁷⁴.

Michael et al found that RMS occurred in younger than 21 years in most of the patients⁵¹. Simona Ognjanovic et al found a male predominance in Rhabdomyosarcoma with a male: female ratio of 1.37:1⁷⁶.

Mir Mahmood Seyed Ahadi et al found that mean age at the time of diagnosis of Wilm's tumor was 45.2 months (4years) with a male: female ratio being 1.2:1⁵⁵.

Norman Breslow et al found that median age at diagnosis of Wilm's tumor was 36.5months and 42.5 months for males and females respectively with slight preponderance of females in the National Wilm's tumor study registered between October 1969 and December 1985⁵⁷.

Neuroblastoma develops in younger age group than Rhabdomyosarcoma and ES/PNETs. 50% of cases are diagnosed by the age of 2 yrs and 90% by the age of 5 yrs. Peak age at diagnosis is 18 months⁷⁴. Gregory Hale et al found a slight male predominance with a male to female ratio of 1.3:1²⁸.

Desmoplastic small round cell tumor (DSRCT) occurs in the age group of 15-35 years³⁸. Ordonez NG et al found a male preponderance with a male: female ratio of 4:1⁵⁹.

Small cell Osteosarcoma occurs between 10 to 25 years of age group and also in preschool children with slight male predominance (1.5:1)³⁸. Extraskelatal Mesenchymal Chondrosarcoma occurs between the age group of 15-35 yrs of age with a female preponderance^{38,74}.

Common locations of Malignant Round Blue Cell Tumors

Table-3

Non Hodgkin lymphoma	Lymphnode
Ewing's sarcoma / PNET	Bone, soft tissue
Rhabdomyosarcoma	Soft tissue
Wilm's tumor	Kidney
Neuroblastoma	Adrenals, Sympathetic nervous system
Desmoplastic small round cell tumor (DSRCT)	Retroperitoneum
Small cell Osteosarcoma	Bone
Extraskeletal Mesenchymal Chondrosarcoma	Soft tissue

Clinical features

Non Hodgkin's Lymphoma (NHL)

NHLs present as enlarged non tender lymphnodes (often >2 cm) in more than two third of cases. The remaining one third of NHLs present with symptoms related to the extranodal site⁸³. Antoria M.S.Muller found that extra nodal NHL constitutes 20-30% of all NHL⁴. Philip Saleem et al states that gastro intestinal NHL (49.5%) is the most common extra nodal site followed by Waldayer's ring (19%), bone, skin, breast, ovary, thyroid and parotid⁶³.

Ewing's sarcoma / PNET

These tumors clinically simulate osteomyelitis because of pain, fever and leukocytosis⁴⁹. Kissane et al found that Ewing's sarcoma has a predilection for lower segment of skeleton⁴¹.

Rhabdomyosarcoma (RMS)

Rhabdomyosarcoma arises from various anatomical sites. These tumors present as bulging, infiltrative, growing soft tissue masses that may be fungating, when they present in external locations such as conjunctiva and vagina⁵.

Agarwala et al found that most common site of Rhabdomyosarcoma is head and neck region followed by genitourinary tract and extremities⁶⁹.

Wilm's Tumor

Wilm's tumor presented with abdominal mass, hematuria and pain. Hypertension is present in some cases^{30,38}.

Conditions associated with increased risk of Wilm's Tumor⁶⁵

WAGR syndrome- Wilm's tumor, aniridia, genito urinary anomalies, and mental retardation.

Denys –Drash syndrome

Familial Wilm's Tumor

Perlman syndrome

WT 1 intron 9 splice mutation (Frasier syndrome)

Beckwith-Wiedemann syndrome

Simpson –Golabi-Behmel syndrome

Isolated hemihypertrophy

Bloom syndrome

Trisomy 13, 18

Neuroblastoma

Neuroblastoma usually presents with abdominal mass, fever and weight loss⁴³. DeLorimer et al, based on California Tumor Registry found that most common site of Neuroblastoma was retroperitoneum followed by mediastinum and cervical region. About half of the retroperitoneal tumors arise from the adrenals¹⁷.

Various clinical presentations of Neuroblastoma^{3,55}

1. Watery diarrhea (Kerner –Morrison syndrome)
2. Cushing's syndrome
3. Heterochromia iridis & Horner's syndrome
4. Opsoclonus / myoclonus – Dancing eye movements
5. Beckwith – Wiedemann syndrome
6. Hirschprung's disease
7. Hypertension
8. Blue berry muffin baby – blue red cutaneous metastases

Common Metastatic sites of Malignant Round Blue Cell Tumors

NHL	-	Generalised disease
Ewing's sarcoma	-	Lungs pleura, skull, Central nervous System, rarely lymph node
Rhabdomyosarcoma-		Soft tissue, serosal surfaces, lungs, bone marrow, lymph nodes
Wilm's tumor	-	Lungs, liver, peritoneum, Regional lymph nodes
Neuroblastoma	-	Liver (Pepper syndrome) skeletal system, lymphnode, orbit
DSRCT	-	Liver, pancreas, ovary
Osteosarcoma	-	Bones, Lungs, pleura, heart

GROSS FEATURES

Non Hodgkin's Lymphomas present with generalized lymphadenopathy and hepatosplenomegaly. Nodes are enlarged and have solid homogenous gray white appearance^{38,83}.

Ewing's sarcoma / PNET arise in the medullary cavity and transgress the cortex and periosteum producing soft tissue masses. The tumor is tan white (or) fish flesh like appearance and contains areas of hemorrhage and necrosis^{38,49}.

Rhabdomyosarcomas exhibit different gross characters in different sites. In body cavities, they present as well circumscribed and multinodular polypoid growth with a glistening, gelatinous gray white surface. On cut section they show areas of hemorrhage or cystic change. In deep seated sites they are less well defined with infiltrative pattern of growth. Cut surface is firm and rubbery and have mottled gray white to pink tan appearance⁷⁴.

Wilm's tumors are solitary, well circumscribed, rounded and soft in consistency with the cut surface predominantly solid and often exhibiting cystic change, hemorrhage and necrosis. Solid areas appear pale gray or tan with lobular pattern because of fibrous septation^{8,38}.

Neuroblastomas are lobulated masses with an average diameter of 6-8cm. They are surrounded by delicate membranous capsules. Cut surface shows soft, fleshy, gray, partially hemorrhagic tumor^{38,74}.

DSRCTs are solid, large multiobulated masses, on cross section show white (or) gray white appearance, with cystic change and necrosis^{38,77}.

Small cell Osteosarcoma grossly appears similar to that of conventional Osteosarcoma and present as bony hard to cystic, friable grayish white tumor with areas of hemorrhage^{22,38}.

Extra skeletal Mesenchymal Chondrosarcoma presents as a multiobulated circumscribed mass of variable size. Cut surface show mixture of fleshy gray white soft tissue with irregularly sized cartilage and bony tissue^{38,74}.

Methods in the diagnosis of Malignant Round Blue Cell Tumors⁶²

Table-4

Light microscopy

Electron microscopy

Immunophenotyping (Paraffin sections)

Immunophenotyping (Monoclonal antibodies, frozen sections)

Methods using imprints

PAS and special stains

Immunophenotyping (Polyclonal and monoclonal antibodies)

Interphase cytogenetics

Methods using viable tumor tissue

Short term tissue culture

Cytogenetics and molecular cytogenetics (FISH)

Molecular genetic analysis

Biochemical analysis

Neurotransmitter analysis (Homovanillic acid, vannillyl mandelic acid)

MICROSCOPIC FEATURES

In hematoxylin and eosin sections, diagnosis is by cytoplasmic and nuclear features with the pattern of arrangement including the intervening stromal cells.

NON HODGKINS LYMPHOMA (NHL)

Nodal

The lymph node structure shows total or partial effacement of architecture and composed of monotonous population of small lymphocytes with clumped chromatin, inconspicuous nucleoli and scanty cytoplasm^{38, 77}.

Extra nodal

NHL can also be seen in extra nodal sites in relation to mucosae or glandular epithelia, such as gastrointestinal tract, salivary glands, lacrimal glands, lung, thyroid, conjunctiva, bladder and skin^{8, 38}.

EWING'S SARCOMA / PNET

The tumor cells are small and uniform with indistinct cell borders resulting in a syncytial appearance. The tumor cells have scanty clear cytoplasm, round nuclei with frequent indentation and small nucleoli. They are arranged in solid sheets, around the blood vessels (Pseudo-rosette) and also as true rosettes^{38, 77}.

EMBRYONAL RHABDOMYOSARCOMA

This tumor is characterized by alternating densely packed hypercellular and loosely textured myxoid areas. The tumor cells are small round or spindle shaped with hyperchromatic nuclei, 1 or 2 nucleoli with indistinct cytoplasm.

There are variable amounts of large cells with abundant eosinophilic cytoplasm and round vesicular nuclei (Rhabdomyoblast)⁷⁴.

BOTRYOID RHABDOMYOSARCOMA

This tumor is characterized by a subepithelial condensation of tumor cells separated from an intact surface epithelium by a zone of loose stroma (Nicholson's cambium layer)^{38,74}.

ALVEOLAR RHABDOMYOSARCOMA

The tumor cells are small, round to oval cells with scanty cytoplasm and round nuclei. The tumor cells are separated into nests by fibrous septa with central loss of cohesion and formation of irregular alveolar spaces⁷⁴.

WILM'S TUMOR

This tumor usually presents with triphasic components and sometimes biphasic or monophasic. The triphasic components composed of combinations of epithelial, stromal and blastemal elements. Epithelial components are characterized by embryonic tubular structures resembling normal developing tubules and glomeruli. . Mesenchymal elements are spindle shaped fibroblast like cells with a smooth muscle and skeletal muscle differentiation.

Blastemal components are cellular areas composed of small round to oval primitive cells with scanty cytoplasm, round to oval nuclei, coarse chromatin arranged in diffuse sheets and nests^{38,77}.

NEUROBLASTOMA

The tumor cells are small and regular with round deeply staining nuclei, slightly larger than lymphocytes. There is little cytoplasm with poorly defined cytoplasmic borders. The tumor cells are separated into small lobules by delicate fibrovascular septa. Homer-Wright rosettes are central fibrillary material surrounded by tumor cells and are seen in 1/4 to 1/3 of cases. Calcification may appear as a dust like basophilic stippling, as closely aggregated round concretions in a “chicken wire” fashion^{38, 74}.

DESMOPLASTIC SMALL ROUND CELL TUMORS (DSRCTs)

DSRCTs composed of sharply outlined islands of tumor cells separated by abundant cellular stroma (desmoplastic). The tumor cells are small round, monotonous with scanty cytoplasm and hyperchromatic nuclei^{8, 38}.

SMALL CELL OSTEOSARCOMA

The tumor cells are round to spindle shaped with scanty cytoplasm and round hyperchromatic nuclei arranged in diffuse sheets with intervening neoplastic osteoid^{22, 38, 49}.

MESENCHYMAL CHONDROSARCOMA

Tumor exhibits bimorphic pattern, composed of sheets of undifferentiated small, round to oval cells with islands of cartilaginous area. The cartilaginous foci are usually well defined with central calcification and ossification⁷⁴.

MYXOID CHONDROSARCOMA

The tumor cells are round to elongated with uniform size and shape. They have deeply eosinophilic cytoplasm and small round hyperchromatic nuclei. They are arranged in anastomosing cords, strands and pseudoacinar pattern with the intervening stroma composed of mucoid material^{38, 74}.

POORLY DIFFERENTIATED SYNOVIAL SARCOMA

These are highly cellular with numerous mitoses and necrosis. The tumor cells are darkly staining ovoid to round cells similar to that seen in other small round cell tumors⁷⁴.

ROUND CELL LIPOSARCOMA

The tumor is characterized by small, round cells with acidophilic cytoplasm and round vesicular nuclei arranged in nests, sheets and pseudoglandular pattern with typical lipoblasts⁷⁴.

MALIGNANT RHABDOID TUMOR

The tumor cells are round to oval with eosinophilic cytoplasm and round vesicular nuclei arranged in diffuse sheets, alveolar and trabecular pattern. The tumor cells have large cytoplasmic, eosinophilic hyaline globules displacing the nuclei laterally resulting in a plasmacytoid appearance^{38, 74}.

CYTOLOGICAL FEATURES

Table-5

Features	Tumor Type
Uniform round cells	Ewing's sarcoma, Neuroblastoma, Alveolar RMS
Spindle shaped (or) fusiform cells	Embryonal RMS, Ewing's Sarcoma /PNET, Wilm's tumor
Rim of basophilic cytoplasm	Burkitt's NHL
Thin rim of clear cytoplasm due to prominent glycogen content	Ewing's Sarcoma /PNET
Vacuoles in cytoplasm	Burkitt's NHL
Convolutated nuclei	Lymphoblastic NHL
Peripheral nucleoli	Large cell NHL
Central prominent nucleoli	Immunoblastic NHL
Thin rim of cytoplasm with glycogen positivity	Rhabdomyosarcoma, Ewing's Sarcoma /PNET

Presence of differentiating cells in the tumor tissue, which give an important clue to arrive at a diagnosis.

- a) Ganglion cells: Cells with vesicular nucleus, small nucleolus and discernible cytoplasm in Neuroblastoma^{38, 74}.
- b) Rhabdbomyoblasts and strap cells: These are spindle shaped cells with eosinophilic cytoplasm, one (or) two centrally placed nuclei, and prominent nucleoli with (or) without cross striations. This is one of the diagnostic clues for Rhabdomyosarcoma⁷⁴.

c) Spider cells (or) multivacuolated cells: When the glycogen is removed during fixation, it results in large multivacuolated cells with narrow strands of cytoplasm connecting the center of the cell with its periphery. This is also seen in certain cases of Rhabdomyosarcoma⁷⁴.

The centrally located nuclei and the irregular shape of cytoplasmic vacuoles help to distinguish these cells from the more rounded lipid filled vacuoles of lipoblasts.

STROMAL CELLS IN SELECTIVE TUMORS

In certain Malignant Round Blue Cell Tumors, characteristic stromal background helps us to arrive at a diagnosis which is tabulated in the Table No.-6.

Table No- 6

Tumors	Characteristic background
Neuroblastoma	Neurofibrillary background (Mats of neurophil, which are tangled networks of cell processes)
Ewing's sarcoma/PNET	Bicellular strands of tissue separated by 'filmy' vascular stroma, referred as 'filigree' pattern
Desmoplastic Small Round Cell Tumor (DSRCT)	Nests of tumor cells surrounded by abundant fibrous connective tissue
Extraskeletal Mesenchymal Chondrosarcoma	Tumor cells admixed with cartilaginous tissue
Myxoid Chondrosarcoma	Myxoid background
Small cell Osteosarcoma	Malignant osteoid

Radiographic findings in certain Malignant Round Blue Cell Tumors

NHL	CT examination of the thorax and abdomen is a vital element in the assessment of extensions. For stage I or II lesions, MRI of the bone marrow, liver and spleen are done to confirm whether the lesions are localized ¹² .
Ewing's/PNET	This tumor typically presents in diaphyseal or metadiaphyseal region of long bone. The lesion is intramedullary and is associated with cortical thickening and cyclical periosteal reaction, giving rise to the characteristic onion –skin appearance ^{12,49} .
Rhabdomyosarcoma	Radiography is done to confirm the presence of mass whenever there is clinical suspicion. And it may identify calcification and adjacent skeletal involvement ¹² .
Wilms tumor	Intravenous pyelogram shows intrarenal mass that displaces and distorts the pelvis. Computed tomography (CT) and Magnetic resonance imaging (MRI) are used to define the tumor extent ³⁸ .

Neuroblastoma - When it occur in the retroperitoneum, causes anterior, lateral and downward displacement of kidney without hydronephrosis. 50% of Neuroblastoma show fine stippled calcification. Radiolabelled Metaiodobenzylguanidine (MIBG) is incorporated into catecholamine secreting cells to detect bone and soft tissue involvement³⁸.

Special stains

Reticulin stain shows fine branching Reticulin network with pericellular fibrils characteristic of Lymphoma^{29, 37}.

Ewing's sarcoma/PNET usually contains large amounts of cytoplasmic glycogen which is demonstrated by PAS (Periodic Acid Schiff's) stain with diastase control^{29, 37, 38}.

IMMUNOCYTOCHEMISTRY IN DIAGNOSIS OF MALIGNANT ROUND BLUE CELL TUMORS

The development of sensitive reagents and detection systems, together with the introduction of heat-induced antigen retrieval, has rapidly entrenched immunohistochemistry as an indispensable adjunct to routine histological examination, contributing to diagnosis, prognosis and treatment¹.

Immunohistochemistry can be helpful in narrowing the differential diagnosis of small-round-cell tumors.

For example, the cell surface glycoprotein p30/32^{MIC2} is highly expressed in the Ewing family of tumors and also in Rhabdomyosarcoma and Non-Hodgkin's Lymphoma. Antibodies to desmin can be used to distinguish Rhabdomyosarcoma from Ewing's sarcoma, Neuroblastoma, and Lymphoma. Similarly, antibodies to leukocyte common antigen (LCA) can be used to separate hematolymphoid malignancies from the remainder of small-round-cell tumors. Nevertheless, there is no antibody specific for a single tumor type^{2, 5, 40, 50,66,72,81}.

Overlaps of mesenchymal, epithelial, and neural markers are present in a variety of tumors. Furthermore, reactivity to antibodies can vary depending on the preparation of the specimen, the antibody used, and the degree of tumor differentiation.

INITIAL ANTIBODY PANEL¹³

Myogenin or Desmin

NB84 or Neuron specific Enolase

Leukocyte Common Antigen (LCA-CD 45)

CD99

Vimentin

Cytokeratin

The immunocytochemical profiles of conventional and extended spectrums of Malignant Round Blue Cell Tumors and aberrant immunoreactivity of these tumors are presented in Table No.7^{20, 21,42,46,60}.

ANTIBODIES AND IMMUNOREACTIVITY OF MALIGNANT ROUND BLUE CELL TUMORS^{10,13,26,29,38,49,77}

Table No-8

Antibody	Tumors Typically Immunoreact with Antibody	Tumors may Immunoreact with Antibody
Leukocyte common Antigen	Lymphoma	Mesenchymal Chondrosarcoma
S-100 Protein	Neuroblastoma, MPNST Ewing's sarcoma /PNET	DSRCT, Hepatoblastoma, Wilms Tumor, Synovial sarcoma, Rhabdomyosarcoma Myofibroma, Mesenchymal Chondrosarcoma, small cell Osteosarcoma
NB84	Neuroblastoma	DSRCTs, Ewing's sarcoma
Neuron specific Enolase	Neuroblastoma, DSRCT	Rhabdomyosarcoma, Ewing's sarcoma
Myogenin	Rhabdomyosarcoma	DSRCTs, Wilm's Tumor
Desmin	Rhabdomyosarcoma, DSRCT	Wilm's Tumor / MPNST, Rhabdoid Tumor
Muscle specific Actin	Rhabdomyosarcoma	DSRCTs, Wilm's tumor, Rhabdoid tumor, MPNST
CD99	Ewing's Sarcoma /PNET	Leukemia, Lymphoma, RMS, DSRCTs, Synovial sarcoma, Mesenchymal Chondrosarcoma
Pancytokeratin	Synovial sarcoma, Hepatoblastoma, Carcinoma, Rhabdoid Tumor, Germ cell tumors, DSRCT	Ewing's sarcoma, RMS, Leukemia, lymphoma

Antibody panels for defining origin of undifferentiated Malignant

Round Blue Cell Tumors^{8, 13, 38, 77}

Myogenic:	Desmin
	Myogenin
	Muscle Specific Actin
Neural:	Chromogranin
	NB84
	Neuron specific enolase
	S100
Lymphoid:	Leukocyte Common Antigen
	Myeloperoxidase protein
Germ Cell:	Alpha fetoprotein
	Pancytokeratin
Neural Crest:	S100 Protein
	HMB-45
	CD99
Mesenchymal:	Vimentin
	Smooth muscle Actin

ELECTRON MICROSCOPIC FEATURES OF MALIGNANT ROUND BLUE CELL TUMORS,

Triche et al commented that "Overall, electron microscopy is probably the most universally useful of all diagnostic techniques other than light microscopy in round cell tumors." With these tumors, electron microscopy demonstrated itself to be more reliable than immunohistochemistry.

Electron microscopy offers not only greater sensitivity and specificity, but also greater versatility. The concept of replacing electron microscopy with a battery of immunostains has often been advocated as an economic measure^{26,27,33,52,54},

Electron microscopic findings of certain tumors are tabulated in Table No-9

Table No-9

Tumor type	Cytoplasmic Glycogen	Basal lamina	Intercellular junctions	Myofilament	Neurosecretory granules
Ewing's sarcoma/ PNET	+	--	+	--	+
Rhabdomyosarcoma	+	+	--	+	--
Neuroblastoma	--	--	+	--	+
NHL	--	--	--	--	--

Presence of neurosecretory granules in Ewing's sarcoma/PNET favours neuronal differentiation^{16,38,74}

Alternative thin and thick bands of myofilaments are diagnostic of Rhabdomyosarcoma⁷⁴.

Wilm's tumor shows triphasic components. Blastemal components have junctional complexes similar to other epithelial cells. A definite basement membrane was absent but numerous mitochondria, lipid droplets, granular endoplasmic reticulum and Golgi vesicles seen⁸⁵.

MOLECULAR PATHOLOGY OF MALIGNANT ROUND BLUE CELL TUMORS

Molecular markers have been increasingly used as a diagnostic tool as well as indicators for prognosis. However, accurate karyotyping of solid tumors is technically difficult, and successful cytogenetic analysis can be performed in only a subset of cases⁸¹. Despite the technical limitations, detection of a cytogenetic abnormality can be an important diagnostic aid in some childhood cancers⁶⁸. For example, the t (11; 22)(q24;q12) translocation is frequently seen in the Ewing family of tumors, which includes Ewing's sarcoma, peripheral neuroectodermal tumors (PNET), and Askin's tumor. However, this translocation is not specific for the Ewing's family of tumors^{64,58}.

Recent studies have demonstrated that the t(11;22) (q24;q12) translocation can be identified in some of the cases of Neuroblastoma and Rhabdomyosarcoma. Molecular markers that are used to diagnose Malignant Round Blue Cell Tumors are shown in Table No.10^{9,34,44}

MOLECULAR MARKERS USED IN DIAGNOSIS & PROGNOSIS OF MALIGNANT ROUND BLUECELL TUMORS

Table No-10

Tumor	Marker	Locus/Translocation	Comments/Techniques
NB	MYCN	2p24	Amplification (> 10 copies), poor prognosis, usually associated with high stage disease; PCR or southern blot.
NB	Del 1p	1p31-1p32	Associated with high stage disease; smaller deletion associated with more favourable outcome; PCR
ES/PNET	EWS/FLI-1	(11,22)(q24;q12)	Found in up to 90% cases; Types I and II fusion products most common; Type I more favourable; RT-PCR
ES/PNET	EWS/ERG	(21,22)(q22;q21)	Found in up to 5% of cases; RT-PCR
NHL, B-Cell type	IgH	14q32	Gene rearrangements identified in > 90% of cases; PCR or southern blot
NHL, T-Cell type	TCR or	7q34 or 7p15	Rearrangements identified in majority of cases; PCR or Southern blot
Wilms Tumor	WT1	11p13	Mutations occur in both sporadic and hereditary Wilms Tumors; PCR

Tumor	Marker	Locus/Translocation	Comments/Techniques
DSRCT	EWS/WT-1	(11,22) (q113; q12)	Only found in DSRCT; RT-PCR
Alveolar Rhabdomyosarc oma	PAX3/FKHR	(2; 13)(Q35; q14)	Found in up to 70% of cases; 20% cases have t(1;13)(p36;q14), PAX7/FKHR associated with better prognosis; RT-PCR
Synovial sarcoma	SYT/SSX	(X:18)(P11:Q 11)	80% of cases; SYT/SSX1 in biphasic sarcomas. SYT/SSX2 in monophasic type associated with better prognosis: RT-PCR

MATERIALS AND METHODS

The present study had been carried out in the Department of Pathology, Madurai Medical College, Madurai for a period of 2 years from June 2008 to May 2010. The specimens were received from Government Rajaji Hospital and Meenakshi Mission Hospital which cater the health need of the people in and around Madurai.

The clinical details of the patients such as age, sex, symptoms, status of liver, spleen, peripheral smear findings and radiological findings were recorded with the informed consent. The working proforma was appended in annexure I.

The histopathology specimens were fixed in 10% formalin. The entire small biopsy specimens received were embedded. In large specimens, the gross characteristic of the tumor such as size, shape, cut surface, hemorrhage and necrosis were noted. Adequate bits were taken from different areas of the specimen. The tissues were processed, paraffin blocked, 5 micron thin sections were cut and stained with Hematoxylin and Eosin (H &E). Special stains such as PAS (Periodic Acid Schiff's) and Reticulin were used as and when required. Immunohistochemical studies were done in relevant cases. The procedures are shown in Appendix –II. Photographs of the specimen and photomicrographs of the slides were taken whenever needed.

The results of histopathological study of H&E stained sections, special stains and Immunohistochemical results were entered in the proforma and master chart (Annexure IV). The Immunohistochemical marker study had enabled to categorize the Malignant Round Blue Cell Tumors and helped to achieve a more accurate and precise diagnosis there by aiding the clinicians for the management of these patients by more specific therapeutic protocols.

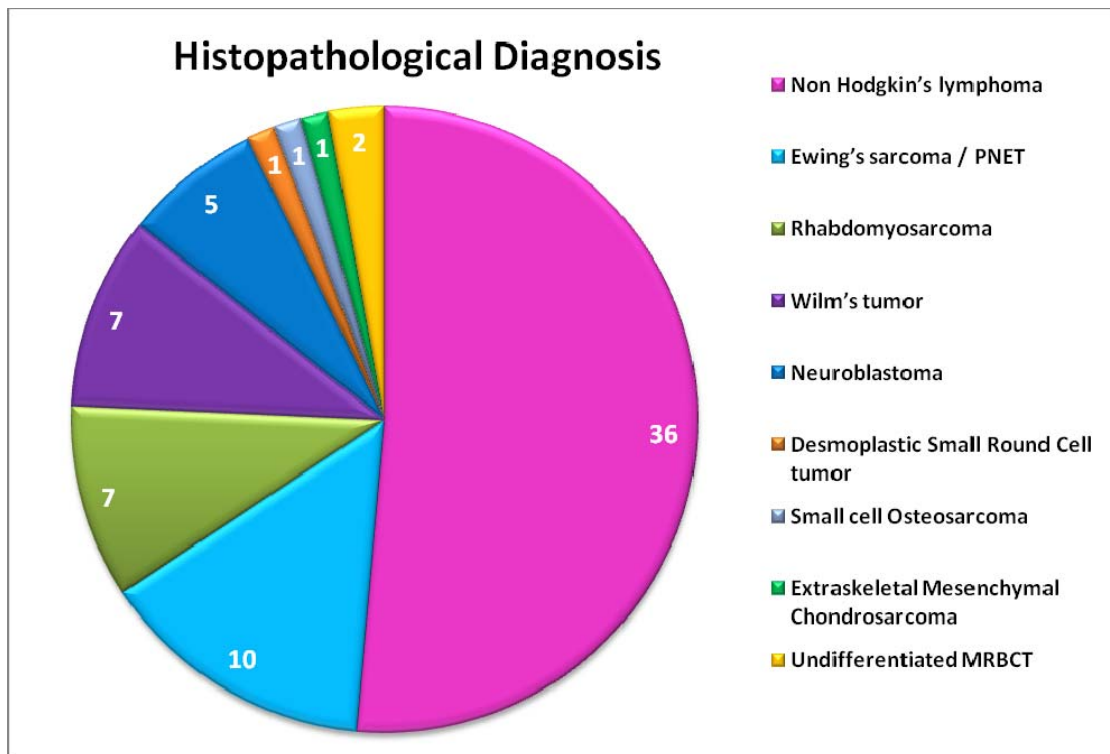
Data analysis was done with the help of computer using **Epidemiological Information Package (EPI 2008)** developed by Center for Disease Control, Atlanta. Using this software, frequencies, percentages, means and standard deviations were calculated.

OBSERVATION AND RESULTS

In the two year study period from June 2008 to May 2010, specimens were received for histopathological examination from Government Rajaji Hospital and Meenakshi Mission Hospital, Madurai.

Out of 17,782 cases from Government Rajaji Hospital 2826 were reported as malignant lesions and out of 7853 cases from Meenakshi Mission Hospital 2210 were reported as malignant lesions. Altogether 70 cases had been reported as Malignant Round Blue Cell Tumor with the incidence of 1.38 %.

ChartNo.1



Among the 70 cases of Malignant Round Blue Cell Tumors studied, Non Hodgkin's Lymphoma was diagnosed in 36 (51.5%) cases, Ewing's sarcoma/PNET in 10 (14.5%) cases, Wilm's tumor and Rhabdomyosarcoma each in 7 (10% + 10%) cases and Neuroblastoma in 5 (7%) cases. The distributions of Malignant Round Blue Cell Tumors were represented by Chart No.1 and Table No.11.

Table No.11 Histopathological Diagnosis

Tumor type	No. of cases	Percentage (%)
Non Hodgkin's lymphoma	36	51.5
Ewing's sarcoma / PNET	10	14.5
Rhabdomyosarcoma	7	10
Wilm's tumor	7	10
Neuroblastoma	5	7
Desmoplastic Small Round Cell tumor	1	1.4
Small cell Osteosarcoma	1	1.4
Extraskelatal Mesenchymal Chondrosarcoma	1	1.4
Undifferentiated Malignant Round Blue Cell Tumor (MRBCT)	2	2.8
Total	70	100

Age incidence

The mean age at diagnosis of Malignant Round Blue Cell Tumors has been tabulated in Table No 12.

Table No.12 : Mean Age at Diagnosis of Malignant Round Blue Cell Tumors

HPE Diagnosis	Age in years	
	Mean	Standard Deviation
NHL (36)	44.9	23
Ewing's Sarcoma (10)	21.3	12.9
Rhabdomyosarcoma (7)	16.9	12.3
Wilm's Tumour (7)	4.5	4.4
Neuroblastoma (5)	13.9	21.3
Malignant round blue cell tumour (2) (Undifferentiated)	30.3	39.2
Others(3)	12.8	12.9

Non Hodgkin's Lymphoma was diagnosed in youngest age of 4 years and oldest age of 92 years.

Ewing's sarcoma/PNET was diagnosed at the youngest age of 8 years and oldest age of 48 years.

Embryonal Rhabdomyosarcoma was diagnosed at the youngest age of 3 months and oldest age of 21 years.

Wilm's tumor was diagnosed at the youngest age of 6 months and oldest age of 12 years.

Neuroblastoma was diagnosed at the youngest age of 3 years and oldest age of 52 years.

Among the 36 cases of Non Hodgkin's lymphoma, 5 cases were diagnosed in pediatric population and the rest 31 cases in adult population especially in patients above 40 years.

Out of 10 cases of Ewing's sarcoma /PNET studied, 1 case was diagnosed in 8 year old child and rest of the cases were diagnosed in adolescent and adult age group.

All the 7 Wilm's tumor cases were diagnosed in children only. All cases of Neuroblastoma occurred in children except one in adult.

4 cases of Embryonal Rhabdomyosarcoma were seen in children and the rest 3 in adults.

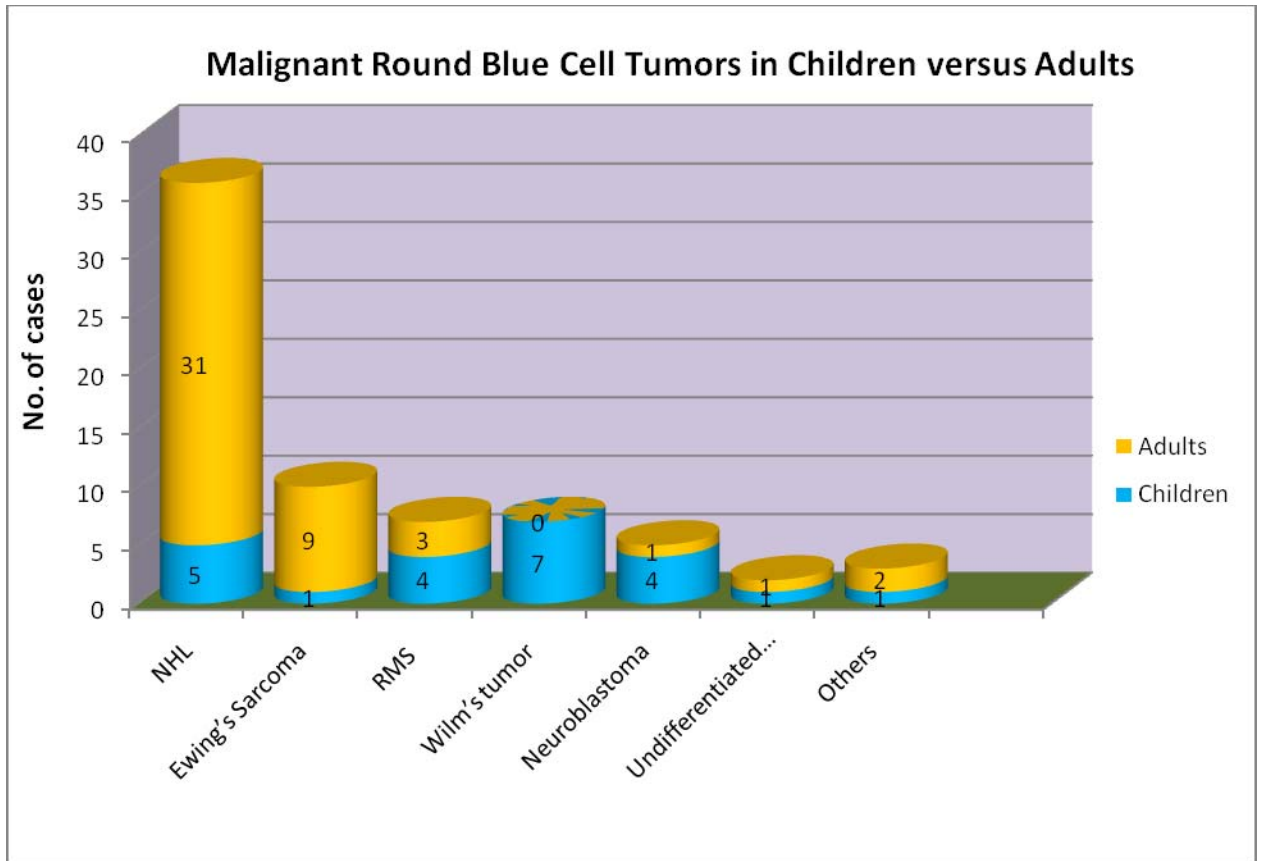
The age incidence of Malignant Round Blue Cell Tumors ranges from 3 months to 92 years in this study. The age incidences of Malignant Round Blue Cell Tumors are shown in Table No 13 and Chart No 2

Malignant Round Blue Cell Tumors in Children versus Adults

Table No -13

Tumor Type	Cases			
	Children		Adults	
	No.	%	No.	%
Non Hodgkin's Lymphoma (36)	5	13.9	31	86.1
Ewing's Sarcoma(10)	1	10	9	90
Rhabdomyosarcoma (7)	4	57.1	3	42.9
Wilm's tumor(7)	7	100	-	-
Neuroblastoma(5)	4	80	1	20
Undifferentiated Malignant Round Blue Cell Tumors (2)	1	50	1	50
Others(3)	1	33.3	2	66.7
Total (70)	23	32.9	47	67.1

Chart No .2

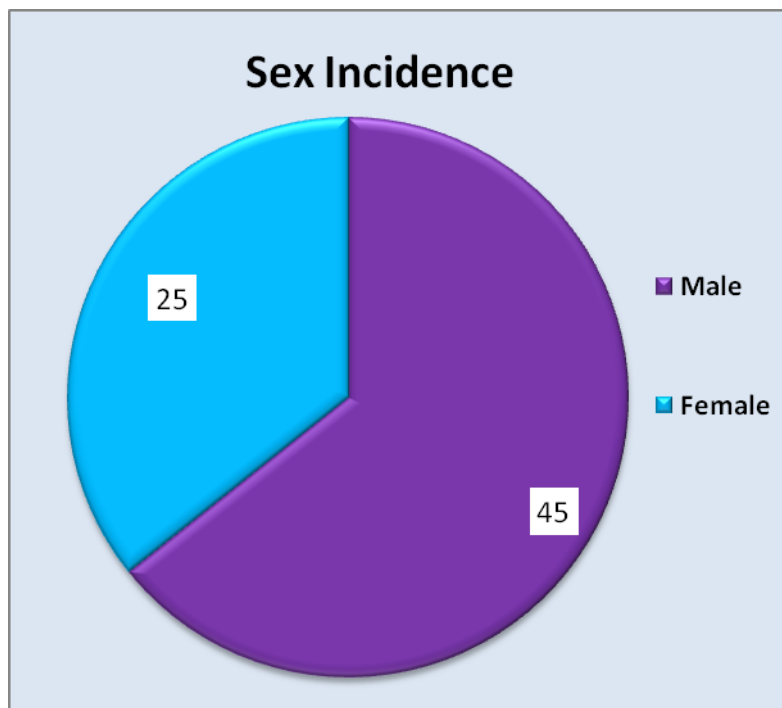


It is observed from the study that Malignant Round Blue Cell Tumors are commonly seen in paediatric age group except Non Hodgkin's Lymphoma which is common in adults

Sex Incidence

Out of the 70 cases of Malignant Round Blue Cell Tumors, 45 cases occurred in males and 25 cases in females with the male to female ratio of (1.8:1) as illustrated in the Chart No 3.

Chart No -3



The incidence of various tumor types according to sex had been depicted in Table No-14 and Chart No-4. Non Hodgkin's Lymphoma predominantly occurred in males with the male to female ratio of 2.3:1.

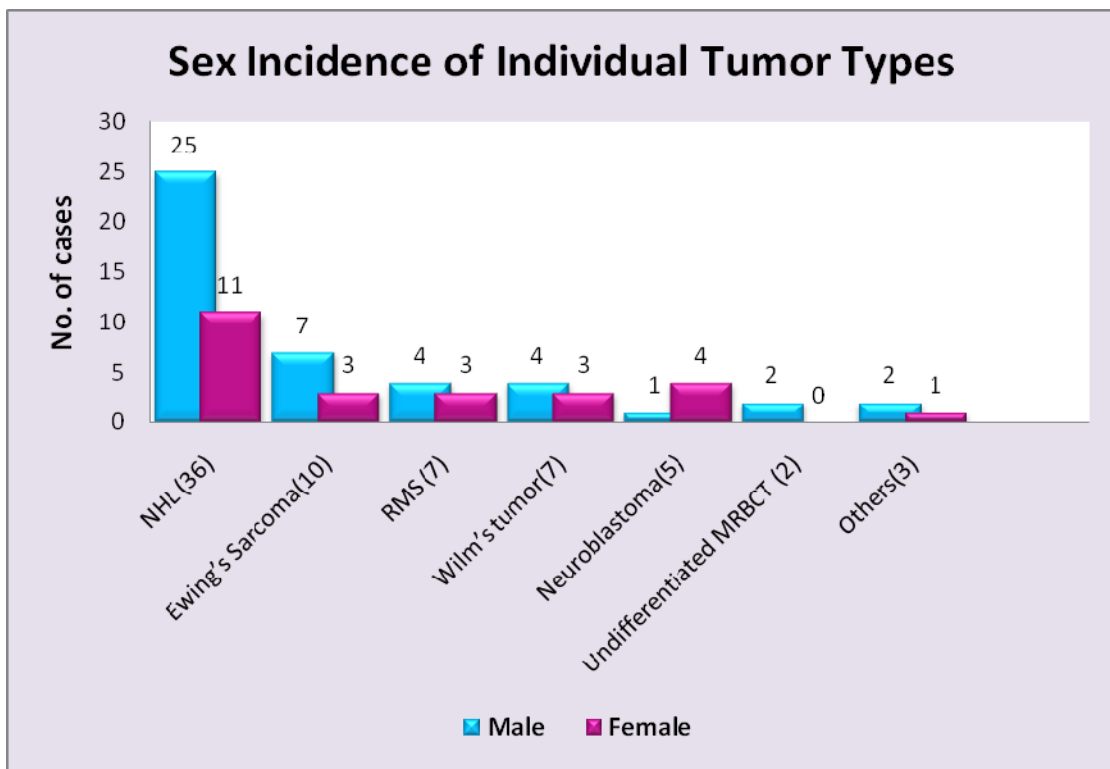
Sex incidence of individual tumor type

Table No-14

HPE Diagnosis	Sex			
	Male		Female	
	No.	%	No.	%
Non Hodgkin's Lymphoma (36)	25	69.4	11	30.6
Ewing's Sarcoma(10)	7	70	3	30
Rhabdomyosarcoma (7)	4	57.1	3	42.9
Wilm's tumor(7)	4	57.1	3	42.9
Neuroblastoma(5)	1	20	4	80
Undifferentiated Malignant Round Blue Cell Tumor (2)	2	100	-	-
Others(3)	2	63.3	1	33.7
Total (70)	45	64.3	25	35.7

The three tumors, Ewing's sarcoma / PNET, Wilm's tumor and Rhabdomyosarcoma had a male predominance with the male to female ratio of 2.3:1, 1.3:1 and 1.3:1 respectively, whereas Neuroblastoma had a female preponderance with the male to female ratio of 1:4.

Chart No.4



It was observed that the Malignant Round Blue Cell Tumors were common in males than females in this study.

CLINICAL PRESENTATION

Non Hodgkin's Lymphoma (NHL)

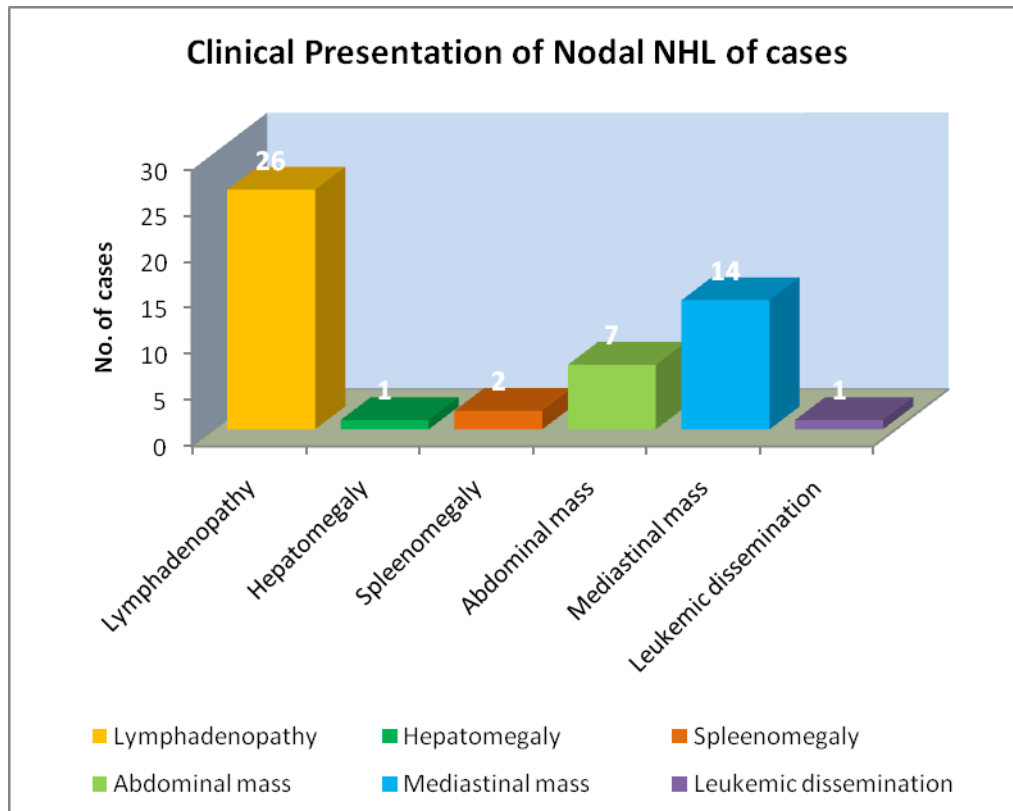
Non Hodgkin's lymphoma was reported in the Nodal (Fig1,2,3) as well as the extra nodal(Fig5,9,13) sites. 26 cases of nodal Non Hodgkin's lymphoma presented with generalised lymphadenopathy and one case had leukemic dissemination as represented in Table No-15 and Chart No -5.

Clinical Presentation of Nodal Non Hodgkin's Lymphoma

Table No-15

Clinical Presentation	No. of cases	Percentage (%)
Lymphadenopathy	26	100
Hepatomegaly	1	3.8
Splenomegaly	2	7.7
Abdominal mass	7	26.9
Mediastinal mass	14	53.8
Leukemic dissemination	1	3.8

Chart No: 5



10 cases of Non Hodgkin's Lymphoma occurred in different extra nodal sites (Fig 6,10,14) with varied clinical presentation depending upon the site of involvement which is shown in the (Table No. 16).

Table No-16

S.No	Site	No of cases	Clinical presentation
1	Parotid	2	Swelling in the parotid region
2	Stomach	3	Epigastric mass
3	Ileum & Colon	2	Right iliac fossa mass
4	Testis	1	Swelling in the scrotum
5	Tibia	1	Swelling above the ankle joint
6	Nasopharynx	1	Dysphagia & Swelling in the neck

Malignant Round Blue Cell Tumors other than NHL

These tumors presented as bony and soft tissue mass in various sites.

Ewing's sarcoma / PNET

Out of 10 cases of Ewing's sarcoma / PNET (Fig 15), 7 were observed in lower segment of the skeleton and the rest 3 in upper segment. The Table No 17 showed various anatomical distribution of these tumors.

Anatomical distribution of Ewing's sarcoma

Table No: 17

S.No.	No. of cases	Anatomical distribution	
		Bone	Soft tissue
1	1	Iliac bone	-
2	3	Tibia	-
3	2	Femur	-
4	1	Fibula	-
5	1	-	Elbow
6	1	-	Chest wall
7	1	-	Axillary region

Wilm's tumor

All the 7 cases presented with abdominal mass and were unilateral.

Rhabdomyosarcoma

The study revealed that Rhabdomyosarcoma (Fig 19) occurred mainly in Head and Neck region and also in other sites such as genitourinary tract and extremities as illustrated in Table No 18

Anatomic distribution of Rhabdomyosarcoma

Table No: 18

S.No.	No. of cases	Clinical presentation
1	2	Extremity
2	2	Nasopharynx
3	2	Genitourinary region
4	1	Eye ball

Neuroblastoma

The study showed that Neuroblastoma(Fig29,30) most commonly presented as retroperitoneal mass rarely as presacral and nasal mass as shown in TableNo19.

Anatomic distribution of Neuroblastoma –Table No: 19

S.No.	No. of cases	Clinical presentation
1	3	Retroperitoneal mass
2	1	Presacral mass
3	1	Nasal mass

Histopathology

70 cases of Malignant Round Blue Cell Tumors were taken for the study. All the sections were stained with hematoxylin and eosin (H & E) stain. Special stains such as Reticulin and PAS were done in relevant cases. Immunohistochemical marker study was also done in selective cases.

Hematoxylin and Eosin (H & E)

The sections stained with H&E were studied under light microscopy and the diagnosis was made based on the cellular morphology such as cytoplasmic and nuclear features, intervening stroma and pattern of arrangement of tumor cells.

The microscopic study showed 34 cases as NHL and 36 cases as Malignant Round Blue Cell Tumors other than NHL. Out of the 34 cases of NHL, 26 occurred in the nodal and the rest 8 in extranodal sites (Fig 7,11,14).

Among the 36 cases of Malignant Round Blue Cell Tumors other than Non Hodgkin's Lymphoma constituted 8 cases of Rhabdomyosarcoma, 7 cases of Wilm's tumor (Fig 25,26,27) , 6 cases of Ewing's sarcoma / PNET and 5 cases of Neuroblastoma. The study also showed one case in each of small cell Osteosarcoma(Fig32), Extra skeletal Mesenchymal Chondrosarcoma(Fig31) and Desmoplastic Small Round Cell Tumor (DSRCT)(Fig 33). Rest of the cases didn't fit into any of the specific type and were categorized as Undifferentiated Malignant Round Blue Cell Tumors.

Special stains

Special stains such as Reticulin and Periodic Acid Schiff's (PAS) were done in selective cases. Reticulin stain (Fig 4) was done for all the cases of nodal NHL which showed fine branching reticulin network with pericellular fibrils characteristic of Lymphoma. PAS stain (Fig 16) was done for 6 cases of Ewing's sarcoma/PNET, which demonstrated abundant cytoplasmic glycogen positivity.

Immunohistochemistry (IHC)

Immunohistochemical marker study was done for all the cases of extra nodal NHL. Specific IHC marker study was done in randomly selected cases to confirm the H &E diagnosis. Panel of IHC marker study was done for all the cases of Undifferentiated Malignant Round Blue Cell Tumors as illustrated in Table No 20.

IHC marker study such as CD 45(Leukocyte common antigen-LCA) (Fig 8,12) was done for all the cases of extra nodal NHL, which showed membranous positivity thus confirming the H&E diagnosis. In addition cytokeratin was also done for gastro intestinal NHL to rule out the possibility of poorly differentiated carcinoma by its negative reaction.

Specific IHC marker study was done for randomly selected 8 cases of Malignant Round Blue Cell Tumors for further evaluation and confirmation. The study confirmed a case of Ewing's sarcoma/ PNET by its membranous positivity for CD99 (Fig17) and positivity for vimentin (Fig18). Two of the Wilm's Tumor cases were confirmed with vimentin (Fig 28) positivity both in blastemal and stromal cells Neuroblastoma was confirmed with positivity for chromogranin (Fig 30) and S-100 in 3 of the cases.

Among the Rhabdomyosarcoma (Fig 20) cases subjected to IHC marker study, one was confirmed with desmin (Fig 21) positivity and another showed negativity for desmin. Hence this case was included in the Undifferentiated Malignant Round Blue Cell Tumors category and subjected to panel of IHC marker study.

Panel of IHC marker study was done for all the Undifferentiated Malignant Round Blue Cell Tumors. Vimentin and CD 99 were positive in 4 cases and proved to be Ewing's sarcoma /PNET. Two cases showed CD 45 positivity and were included in extranodal Non Hodgkin's Lymphoma category. The rest two cases (Fig 34) showed negativity for all the IHC markers in the primary antibody panel. The final results were tabulated in the Table No- 21.

The histopathological diagnosis based on H&E sections categorized the 70 cases of Malignant Round Blue Cell Tumors into 34 cases of NHL (26 nodal+ 8 extranodal) and 36 cases of Malignant Round Blue Cell Tumors other than NHL.

Among the 36 cases of Malignant Round Blue Cell Tumors other than NHL 2 cases had been diagnosed as NHL by Immunohistochemical marker study, thereby making a total of NHL as 36 cases. Two cases remained in the undifferentiated category even after Immunohistochemistry.

DISCUSSION

Malignant Round Blue Cell Tumors comprise a diverse group of diagnostically challenging primitive and undifferentiated neoplasms. Although classical histological features are highly suggestive of a tumor type, on occasion these tumors are indistinguishable by light microscopy, making a definitive diagnosis difficult. Accurate diagnosis of Malignant Round Blue Cell Tumors has become increasingly crucial, as disparate approaches to therapy are used for distinct tumor types.

Incidence

The incidence of Malignant Round Blue Cell Tumors in the present study is 1.38%. The 70 cases of Malignant Round Blue Cell Tumors were categorized into 36 cases of Non Hodgkin's Lymphoma (NHL) and 34 cases of Malignant Round Blue Cell Tumors other than NHL.

Non Hodgkin's lymphoma is the most common tumor, constituting 36 cases (51.5%). Ewing's sarcoma / PNET constitute 10 cases (14.5%). Wilms tumor and Rhabdomyosarcoma each constitute 7 cases (10%+10%). Neuroblastoma constitutes 5 cases (7%).

Age

In this present study, the age of the patients with Malignant Round Blue Cell Tumors ranged between 3 months to 92 years, 23 cases in children with 32.9% of incidence and 47 cases in adults with 67.1% of incidence.

NHL is common in adults than in children with ratio of 4:1. The mean age at diagnosis of NHL is 44.9 years. Surveillance Epidemiology and End Results⁸⁰ (National Cancer Institute, USA) showed 67 years as the median age at diagnosis of NHL.

Ewing's/PNET is predominantly seen in adolescent age group, which is similar to the study conducted by Carvajal R et al⁶ in which common age group for Ewing's sarcoma / PNET is 10-20 years.

In the present study, Rhabdomyosarcoma (RMS) (Fig 22,23) is common in children than adults. Most of the RMS cases are below 21 years with the mean age at diagnosis of 16.9 years. This study correlates well with the study conducted by Michael et al⁵¹ who also found that RMS occurred commonly in patients younger than 21 years.

This current study showed median age at diagnosis of Wilm's tumor as 53 months (4.5 years). Mir Mahmood Seyed Ahadi et al⁵⁵ found that mean age at the time of diagnosis as 45.2 months (4 years) which is slightly lower than that found in the present study. Norman Breslow et al⁵⁷ found that median age at diagnosis of Wilm's tumor is 39.5 months.

In this study, 80% cases of Neuroblastoma are seen under the age of 6 correlating with the study conducted by Stiller C A et al⁷⁹ which also showed 80% of cases below the age of 4.

Sex

In the present study Malignant Round Blue Cell Tumors show a male: female ratio of 1.8:1.

This study shows a male preponderance of Non Hodgkin's lymphoma with a male: female ratio of 2.2:1, which correlates with the Surveillance Epidemiology and End Results⁸⁰ (National Cancer Institute.USA) which also shows a male preponderance with a male: female ratio of 1.4:1.

Carvajal R et al⁶ found slight male predominance of Ewing's sarcoma/PNET. This current study correlates with the above study with a male: female ratio of 2.3:1.

Simona Ognjanovic et al⁷⁶ found a male preponderance for Rhabdomyosarcoma with a male: female ratio of 1.37:1. This present study also shows a male preponderance with the male: female ratio of 1.3:1.

Mir Mahmood Seyed Ahadi et al⁵⁵ found that Wilm's tumor is common in males with a male: female ratio being 1.2:1. This present study correlates with the above study with a male: female ratio of 1.3:1.

Norman Breslow et al⁵⁷ found that slight preponderance of females in the National Wilm's tumor study registered between October 1969 and December 1985.

Gregory Hale et al²⁸ found a male preponderance in Neuroblastoma with a male to female ratio of 1.3:1. In contrast, this present study shows distinct female preponderance with the male: female ratio being 1:4.

Site

In this study, extra nodal NHL constitutes about 27% of the total NHL cases recorded. This can be compared with Antoria M.S.Muller et al⁴ who found that extra nodal NHL constitutes 20-30% of all NHL in his study.

Sandeep Agarwala et al⁶⁹ found that most common site of occurrence of Rhabdomyosarcoma is the head and neck region. This present study correlates with the above study.

Kissane et al⁴¹ found that Ewing's sarcoma has a predilection for lower segment of skeleton as this current study.

Neuroblastoma commonly present as retroperitoneal mass which correlates with the study conducted by DeLorimer et al¹⁷ based on California Tumor Registry.

ROLE OF SPECIAL STAIN

Reticulin stain was done for all the cases of nodal lymphoma to confirm the effacement of architecture, which is characteristic of Lymphoma.

PAS stain was done to confirm for all the cases of the Ewing sarcoma/PNET to confirm the presence of glycogen in the cytoplasm. Although these two special stains are not specific for the diagnosis they had a limited role in categorizing the Malignant Round Blue Cell Tumors (MRBCT).

ROLE OF IMMUNOHISTOCHEMISTRY

Immunohistochemistry (IHC) must be interpreted in the context of light microscopic findings. In most of the cases, the combination of clinical and radiographic findings with light microscopic appearance is sufficient for the diagnosis. The decision about when to order immunohistochemistry depends upon the complexity of the tumor and the pathologist's experience.

Beginning with the limited antibody panel, helps to refine the differential diagnosis and allows a more systematic use of financial and tissue resources. The panel used depends upon the laboratory resources and capabilities. If results are unexpected (or) contradictory, another more comprehensive panel may be used. The use of panel of IHC markers rather than over-reliance on a single antibody is an important diagnostic principle.

Immunohistochemistry was done for all the cases of extra nodal NHL. All these tumors show membranous positivity of CD45 (Leukocyte Common Antigen-LCA). Cytokeratin was done for gastro intestinal NHL which showed negative reaction thus ruling out the possibility of poorly differentiated carcinoma. So the accurate diagnosis was arrived for extra nodal NHL, which helped the patients to get a specific treatment.

Immunohistochemical marker study was also done for few randomly selected cases of Malignant Round Blue Cell Tumors other than NHL to confirm the H&E diagnosis. The results are shown in the Table No-22

Table No-22

S.No	Diagnosis based on H &E	No of cases	IHC marker
1	Ewing's sarcoma /PNET	1	CD 99+
2	Wilm's tumor	2	Vimentin +
3	Neuroblastoma	3	Chromogranin +
4	Rhabdomyosarcoma	2	Desmin + in 1 case Desmin - in 1 case*

*This case was included in Undifferentiated Malignant Round Blue Cell Tumors and subjected to panel of IHC marker study.

The H&E diagnosis very well correlated with the IHC marker study in all the cases except one. This case was diagnosed as Rhabdomyosarcoma in H&E sections which showed negative reaction for desmin. Hence it is included in the undifferentiated category, which later on proved to be Non Hodgkin's Lymphoma by its CD 45 positivity.

In undifferentiated Malignant Round Blue Cell Tumors, 4 cases showed membranous positivity for CD 99 and diffuse positivity for vimentin which helped to arrive at a specific diagnosis of Ewing's sarcoma/ PNET. The close differential diagnosis for Ewing's sarcoma/ PNET is Rhabdomyosarcoma, Lymphoma and small cell Osteosarcoma.

In the panel of IHC marker (Table No-20) study, negative reaction for desmin and CD 45 helped to exclude the Rhabdomyosarcoma and Lymphoma respectively, whereas the small cell Osteosarcoma is ruled out due to the absence of neoplastic osteoid in H&E sections.

One of the undifferentiated Malignant Round Blue Cell Tumors arising from the bone showed negative reaction for CD 99 and membranous positive reaction for CD 45. Hence this is included in the extra nodal NHL category.

Most of the Malignant Round Blue Cell Tumors are categorized into specific types which enables the patients to get appropriate treatment according to the final diagnosis.

Two of the undifferentiated Malignant Round Blue Cell Tumors showed negative reaction for all the IHC markers in the primary antibody panel and doesn't fit into any of the specific category. So these two cases still remain in the category of Undifferentiated Malignant Round Blue Cell Tumors.

For evaluating these two cases, a more comprehensive panel of IHC markers may be employed to achieve a specific diagnosis. Techniques such as electron microscopy or molecular testing may be necessary to make a diagnosis in these cases. Even though IHC marker study is a valuable tool, but it has significant pitfalls in certain instances, such as inadequate fixation, over fixation, incomplete dehydration during processing or if the paraffin is too hot. These are the instances where antigen may be lost from the tissue and these technical factors are to be considered while interpreting IHC marker study.

For making the diagnosis of Malignant Round Blue Cell Tumors, the combination of clinical history, radiologic features and light microscopic appearance are sufficient in majority of patients. Immunohistochemistry can be employed wherever there is difficulty in making the diagnosis by H&E sections. The availability of more specific and more sensitive IHC markers has enabled to categorize these Malignant Round Blue Cell Tumors. Therapeutic modality varies with the individual tumor. So an exact diagnosis of Malignant Round Blue Cell Tumor is essential to enable the patients to get an appropriate management.

SUMMARY

1. This study was conducted in Madurai Medical College, Madurai from June 2008 to May 2010 with the materials received from Government Rajaji Hospital and Meenakshi Mission Hospital, Madurai during this period.
2. Among the 5036 malignant lesions, Malignant Round Blue Cell Tumors constitute about 70 cases with an incidence of 1.38%.
3. Non Hodgkin's Lymphoma (NHL) is the most common type of tumor in this study which is about 51.5% (36 cases) and Malignant Round Blue Cell Tumors other than NHL constitute 48.5% (34 cases).
4. Extra nodal Non Hodgkin's Lymphoma constitutes about 27% of all the cases of NHL.
5. Malignant Round Blue Cell Tumors other than NHL is common in children and adolescence whereas NHL is common in the older age group.
6. Malignant Round Blue Cell Tumors have a male preponderance with a male to female ratio of 1.8:1, except in Neuroblastoma which is common in females.
7. The common clinical presentation of NHL is lymph nodal enlargement

8. Ewing's sarcoma/PNET affects lower segment of the skeleton whereas Rhabdomyosarcoma involves the head and neck region. Neuroblastoma usually presents as retroperitoneal mass.
9. Reticulin and PAS stains are supportive to the diagnosis of NHL and Ewing's sarcoma /PNET respectively in the study.
10. The Immunohistochemistry has confirmed hematoxylin and eosin (H&E) based diagnosis in most of the cases.
11. The undifferentiated Malignant Round Blue Cell Tumors are categorized into specific types by Immunohistochemical marker study in most of the cases.
12. Specific diagnosis of the Malignant Round Blue Cell Tumors by immunohistochemical marker study enabled the patients to get appropriate treatment.

CONCLUSION

Malignant Round Blue Cell Tumors comprise a wide range of neoplasms varying considerably in regard to etiology, site of origin, involvement of skeletal system and prognosis. But on light microscopy, these tumors have the basic morphologic themes of small round blue cells. In the diagnosis of Malignant Round Blue Cell Tumors, clinical history, radiologic features, light microscopic findings and results of Immunohistochemistry are all taken into consideration. Immunohistochemical marker study has a great role of categorizing these tumors and accurate diagnosis is made possible which enable the patients to get the appropriate treatment.

ANNEXURE – I

PROFORMA

Name :

Age / Sex :

IP No. :

Address :

Complaints :

H/o Present Illness :

Family H/o :

General Examination :

Nutrition

Stature

Pallor

Jaundice

Hepatomegaly

Splenomegaly

Lymphadenopathy

Mediastinal Mass

Abdominal Mass

Peripheral Smear Examination :

Radiological Examination :

Biochemical Examination :

Blood :

Urine :

Morbid Anatomy :

Macroscopy :

Microscopy :

H&E :

Special Stains :

Diagnosis

Immunohistochemistry :

Miscellaneous :

Final Diagnosis :

ANNEXURE - II

PROCEDURES

HEMATOXYLIN AND EOSIN^{29,37}

1. Deparaffinize sections, hydrate through graded alcohols to water.
2. Stain in Ehrlich's Hematoxylin for 30 minutes
3. Wash well in tap water
4. Differentiate in 1% acid alcohol
5. Wash well in tap water until sections are again blue for 10-15 minutes
6. Stain in 1% eosin for 1 to 2 minutes
7. Wash in running tap water for 1 to 5 minutes
8. Dehydrate the alcohols clearly in Xylol and mount in DPX.

SPECIAL STAINS^{29,37}

Periodic Acid Schiff's Stain (Glycogen in ES/PNET)

Method:

1. Dewax sections and bring to distilled water
2. Treat with periodic acid for 5 minutes
3. Wash well with several changes of distilled water
4. Cover with Schiff's solution for 15 minutes
5. Wash in running tap water 5-10 minutes
6. Stain nuclei with Harris Hematoxylin. Differentiate as appropriate
in acid alcohol and blueing in tap water for 5 minutes.
7. Wash in water
8. Rinse in absolute alcohol
9. Clear in Xylene and mount with DPX

RESULTS

Glycogen : Magenta

Nuclei : Blue

I. RETICULIN STAIN^{29,37}

1. Bring sections to water.
2. Treat with potassium permanganate solution for 1-2 minutes.
3. Wash in water, bleach with the potassium metabisulphite solution.
Wash well in water.
4. Treat with the iron alum solution for 1 minute. Wash well in tap
and then distilled water.
5. Treat with ammoniacal silver solution for 1 minute.
6. Wash briefly in distilled water and reduce in 10% formalin for 3
minutes.
7. Wash, then tone in 0.2% gold chloride for up to 10 minutes.
8. Rinse in distilled water, then treat with the potassium
metabisulphite solution for 1 minute.
9. Rinse with distilled water and fix with 5% hypo for 1-2 minutes.
10. Wash, dehydrate, clear, and mount in DPX..

Results:

Reticulin fibers	Black
Nuclei	grey
Collagen	grey-purple

IMMUNO HISTOCHEMICAL STAINING ^{29,37}

1. Sections to alcohol.
2. Block endogenous peroxidase activity by incubating in hydrogen peroxide solution for 30 minutes.
3. Hydrate sections by passing through graded ethanol series and wash in running water for 15 minutes.
4. Incubate sections in the normal swine serum diluted in Tris/saline for 15 minutes.
5. Drain off excess Tris-buffered normal swine serum.
6. Incubate sections with primary antiserum diluted 1:2000, 1:1000, 1:250 and 1:100 in 1% normal swine serum for 30 minutes.
7. Jet wash off excess antiserum and then wash slides in Tris / saline for three 2 minutes changes.
8. Incubate sections in swine anti-rabbit IgG diluted 1:20 for 30 minutes.
9. Jet wash off excess antiserum and then wash slides for three 2 minutes changes.
10. Incubate sections in PAP complex diluted 1:60 in 1% normal swine serum in Tris / saline for 30 minutes.

11. Jet wash off excess complex and wash in Tris / saline for three 2 minutes changes.

12. Incubate sections in DAB medium for 5 minutes.

13. Wash sections in running water for 10 minutes.

14. Counterstain in alum hematoxylin, dehydrate, clear and mount.

Results:

Reaction product	brown
Nuclei	blue

ANNEXURE - III

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ANNEXURE-IV

MASTER CHART

S.No	OP/IP No	HP No	Age	Sex	Liver	Spleen	Adeno pathy	Abdominal Mass	Mediasti nal mass	Peripheral smear	Histopathology	Special stain	IHC	Final Diagnosis by IHC
1	48596	1956/08	3.5yrs	F	--	--	--	+	--	N	Neuroblastoma		chr +	
2	50632	2161/08	60yrs	M	--	--	+	--	--	N	NHL	Reticulin		
3	51948	2236/08	16 yrs	M	--	--	--	--	--	N	Ewings sarcoma	PAS		
4	51090	2346/08	12 yrs	M	--	--	--	+	--	N	Wilms tumor			
5	5252	2675/08	41yrs	M	--	--	+	--	+	N	NHL	Reticulin		
6	68649	3223/08	26 yrs	M	--	--	--	+	--	N	Intra abdominal desmoplastic tumor			
7	67099	3283/08	5 yrs	M	--	--	+	--	--	N	NHL	Reticulin		
8	35280	3434/08	9 yrs	F	--	--	+	--	+	N	NHL	Reticulin		
9	82399	3967/08	48 yrs	M	--	--	+	--	+	N	NHL	Reticulin		
10	83704	3973/08	13 yrs	M	--	--	--	--	--	N	Ewings sarcoma	PAS		
11	85837	3998/08	13 yrs	M	--	--	--	--	--	N	Ewings sarcoma		CD 99 +	
12	84185	4012/08	11 yrs	F	+	+	+	--	+	N	NHL	Reticulin		
13	86281	4482/08	15 yrs	F	--	--	--	--	--	N	Malignant round blue cell tumor	PAS	CD 99+ vimentin+ desmin,chr, LCA -	ewings sarcoma
14	96928	4670/08	4 yrs	F	--	--	--	+	--	N	Wilms tumor			
15	106228	70/09	46 yrs	M	--	--	+	--	+	? CLL/ ? Lmphoma	NHL with luekemic dissemination	Reticulin		
16	101628	117/09	58 yrs	M	--	--	--	--	--	N	Malignant round blue cell tumor		CD 99, vimentin, desmin,chr, LCA -	Undifferentiated malignant round blue cell tumor
17	36768	1193/09	55yrs	F	--	--	+	--	--	N	NHL	Reticulin		
18	31090	864/09	21 yrs	M	--	--	+	--	+	N	NHL	Reticulin		
19	22394	865/09	3 yrs	F	--	--	--	+	--	N	Neuroblastoma			
20	39777	1267/09	55yrs	M	--	--	+	--	--	N	NHL	Reticulin		
21	49232	1665/09	23yrs	F	--	--	+	--	--	N	NHL	Reticulin		
22	53438	2092/09	8yrs	F	--	--	--	--	--	N	Malignant round blue cell tumor	PAS	CD 99+ vimentin+ desmin,chr, LCA -	ewings sarcoma
23	68330	2774/09	52yrs	M	--	--	+	+	+	N	NHL	Reticulin		
24	62623	3001/09	8yrs	M	--	--	--	--	--	N	Wilms tumor			
25	82535	3169/09	3/12mon	M	--	--	--	--	--	N	Embryonal rhabdomyosarcoma		desmin +	

S.No	OP/IP No	HP No	Age	Sex	Liver	Spleen	Adeno pathy	Abdominal Mass	Mediasti nal mass	Peripheral smear	Histopathology	Special stain	IHC	Final Diagnosis by IHC
26	75350	3192/09	38yrs	F	--	--	--	--	+	N	Extra skeletal mesenchymal chondrosarcoma			
27	81871	3318/09	48yrs	M	--	--	--	--	--	N	Ewings sarcoma/PNET	PAS		
28	92630	3697/09	6yrs	F	--	--	--	--	--	N	Rhabdomyosarcoma		TdT ,CD3,CD45+ Desmin--	NHL
29	94621	3733/09	6/12mon	M	--	--	--	+	--	N	Wilms tumor		vimentin +	
30	92905	3743/09	60yrs	M	--	--	+	--	+	N	NHL	Reticulin		
31	98298	3920/09	20 yrs	F	--	--	--	--	--	N	Ewings sarcoma		CD 99 +, vimentin+	
32	106526	255/10	56yrs	M	--	--	--	--	--	N	NHL	--		
33	27290	1304/10	2 1/2 yrs	M	--	--	--	--	--	N	Malignant round blue cell tumor		CD 99, vimentin, desmin,chr, LCA -	Undifferentiated malignant round blue cell tumor
34	22536	1546/10	92 yrs	M	--	--	--	--	--	N	NHL	--	LCA +	
35	5761	439/09	12yrs	M	--	--	--	--	--	N	Small cell osteosarcoma			
36	372587	2249/08	6mon	F	--	--	+	+	--	N	Wilms tumor		chr--	
37	337846	2824/08	54yrs	M	--	+	+	--	+	N	NHL	Reticulin		
38	391309	3170/08	23yrs	M	--	--	+	--	+	N	NHL	Reticulin		
39	379428	2127/10	15 yrs	M	--	--	--	+	--	N	NHL	--		
40	381465	2001/08	15yrs	M	--	--	--	--	--	N	PNET			
41	381804	2056/08	52yrs	M	--	--	--	--	--	N	Olfactory neuroblastoma		chr,S100+ ck--	
42	391882	2130/08	70yrs	M	--	--	+	+	--	N	NHL	--		
43	138828	2161/08	6yrs	F	--	--	--	--	--	N	Neuroblastoma		ck --,vimentin +	
44	141929	3591/08	25yrs	M	--	--	--	--	--	N	Malignant round blue cell tumor		CD 99,vimentin+ desmin,LCA--	ewings sarcoma
45	458851	238/09	35yrs	M	--	--	--	--	--	N	NHL	--	LCA+,ck--	
46	403046	322/09	45yrs	M	--	--	+	--	+	N	NHL	Reticulin	LCA+,ck--	
47	387831	327/09	6yrs	M	--	--	--	+	--	N	Wilms tumor			
48	404170	488/09	60yrs	M	--	--	+	--	--	N	NHL	Reticulin		
49	405561	598/09	5yrs	F	--	--	--	--	--	N	Embryonal rhabdomyosarcoma			
50	408239	879/09	46yrs	M	--	--	+	--	+	N	NHL	Reticulin		
51	408921	922/09	63yrs	M	--	--	+	--	--	N	NHL	Reticulin		
52	412055	1259/09	54yrs	F	--	--	--	+	--	N	NHL	--	ck--,LCA+	
53	412205	1264/09	12yrs	F	--	--	--	--	+	N	Embryonal rhabdomyosarcoma			
54	415344	2442/10	14yrs	F	--	--	--	+	--	N	NHL	--		

S.No	OP/IP No	HP No	Age	Sex	Liver	Spleen	Adeno pathy	Abdominal Mass	Mediasti nal mass	Peripheral smear	Histopathology	Special stain	IHC	Final Diagnosis by IHC
55	405661	1709/09	42yrs	M	--	--	--	--	--	N	Malignant round blue cell tumor	--	CD 99, vimentin, desmin,chr-- LCA +	NHL
56	419067	1950/09	4yrs	F	--	--	--	+	--	N	NHL	--		
57	419608	1659/09	1yrs	M	--	--	--	--	--	N	Embryonal rhabdomyosarcoma			
58	420379	2089/09	21yrs	M	--	--	--	--	--	N	Embryonal rhabdomyosarcoma			
59	420853	2553/09	77yrs	M	--	--	+	--	--	N	NHL	Reticulin		
60	421283	2910/09	70yrs	M	--	--	--	--	--	N	NHL	--		
61	428986	3009/09	1yrs	F	--	--	--	+	--	N	Wilms tumor			
62	429796	3011/09	69yrs	F	--	--	+	+	--	N	NHL	Reticulin		
63	433310	3364/09	21yrs	F	--	--	--	--	--	N	Botryoid variant of embryonal RMS			
64	435413	3547/09	60yrs	F	--	--	+	--	+	N	NHL	Reticulin		
65	435873	3593/09	20yrs	M	--	--	--	--	--	N	Alveolar Rhabdomyosarcoma		desmin+ vimentin,ck --	
66	437342	3725/09	63yrs	M	--	--	+	--	--	N	NHL	Reticulin		
67	438465	s432/10	40yrs	M	--	--	+	--	--	N	Malignant round blue cell tumor		CD99,CD100+ vimentinpatchy + desmin -	ewings sarcoma / PNET
68	439872	s638/10	68yrs	M	--	--	+	--	+	N	NHL	Reticulin		
69	439965	s646/10	45yrs	F	--	--	+	--	--	N	NHL	Reticulin	ck --,CD 45+	
70	455364	G371/10	5yrs	F	--	--	--	+	--	N	Neuroblastoma		ck,desmin,chr-- syn patchy +	
CHR	Chromogranin			LCA	Leukocyte common antigen				CK	cytokeratin		SYN	Synaptophysin	
				+	Positive Finding				--	Negative Finding				



Fig .1: NHL Lymph node: Cut surface is homogenous gray white (864/09)

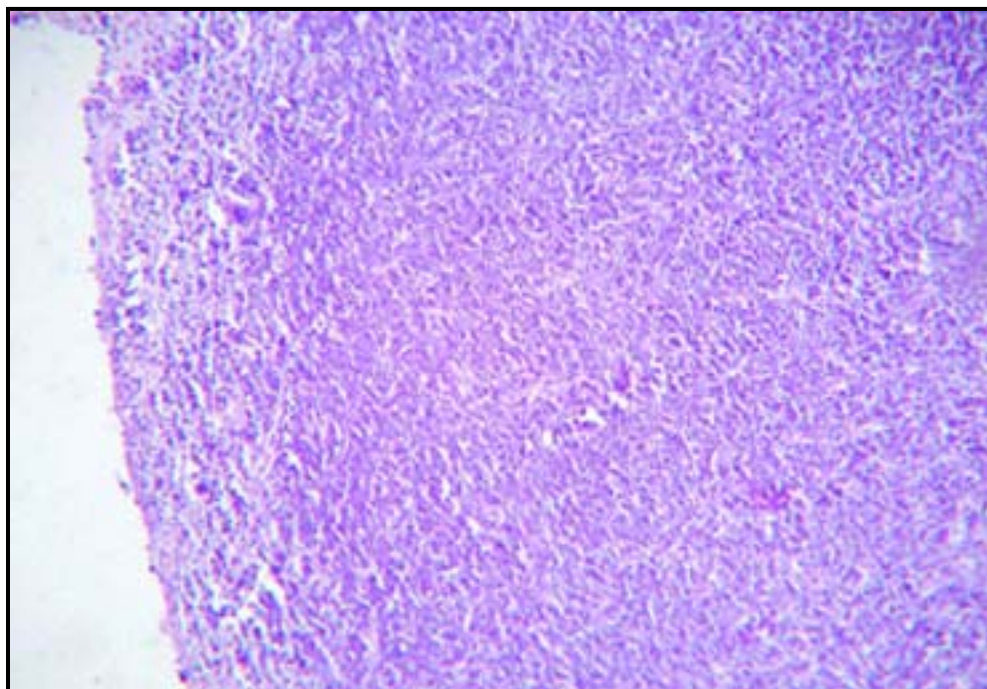


Fig .2: NHL Lymph node: Shows structure of lymph node with effacement of architecture H&EX100 (864/09)

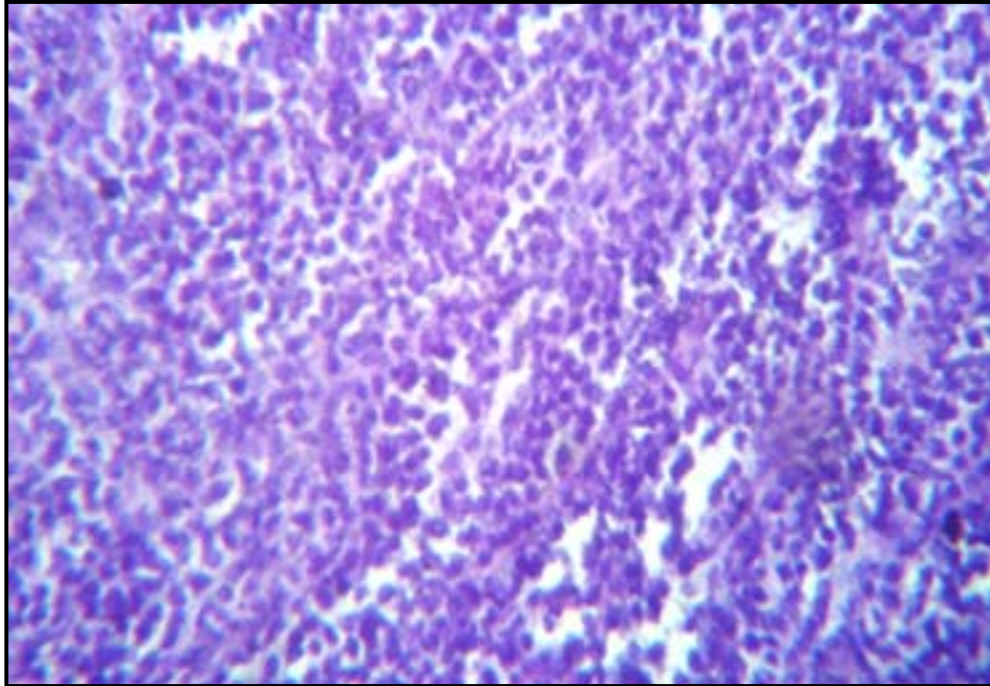


Fig 3: NHL Lymph node: Shows monotonous proliferation of lymphocytes with scanty cytoplasm and regular round nuclei H&E X 400(864/09)

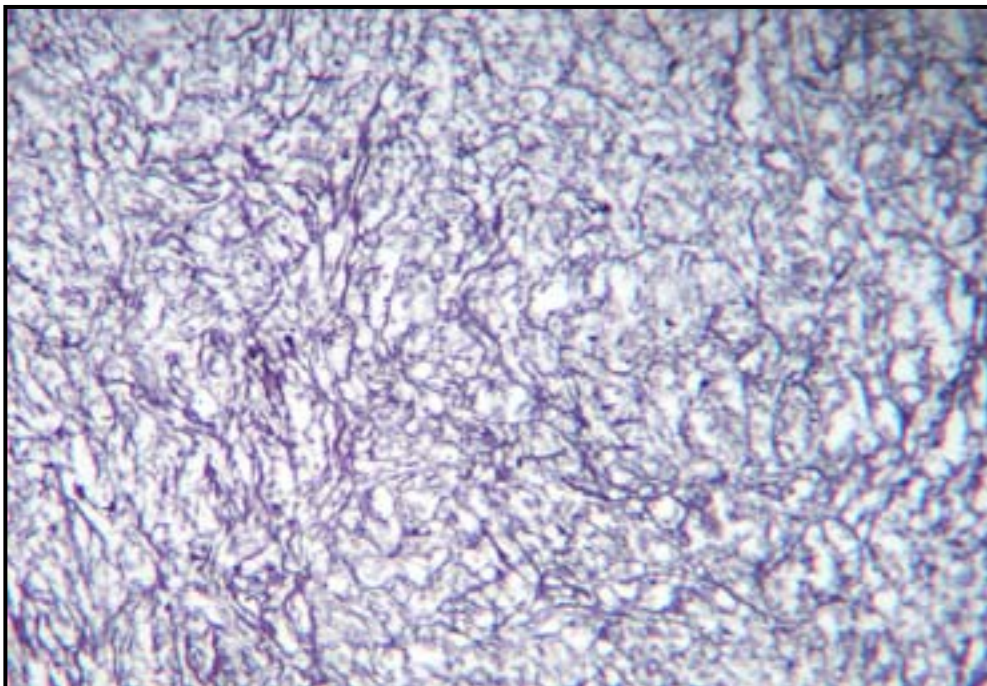


Fig .4: NHL Lymph node shows loss of architecture with pericellular fibrils Reticulin X 400 (864/09)

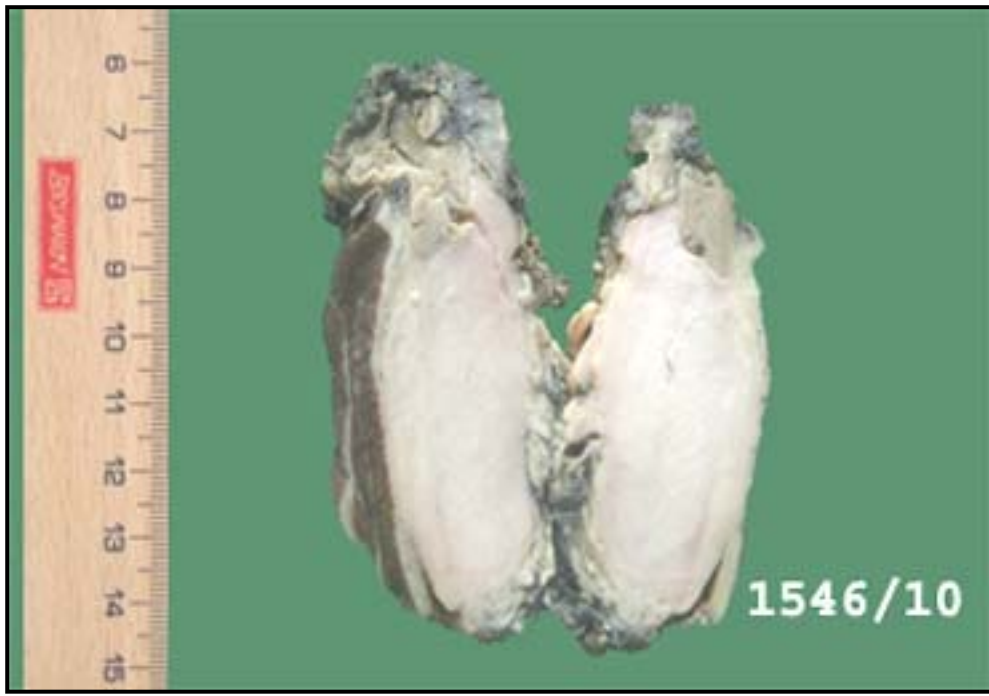


Fig 5: NHL Parotid gland: Shows skin with underlying homogenous gray white mass (1546/10)

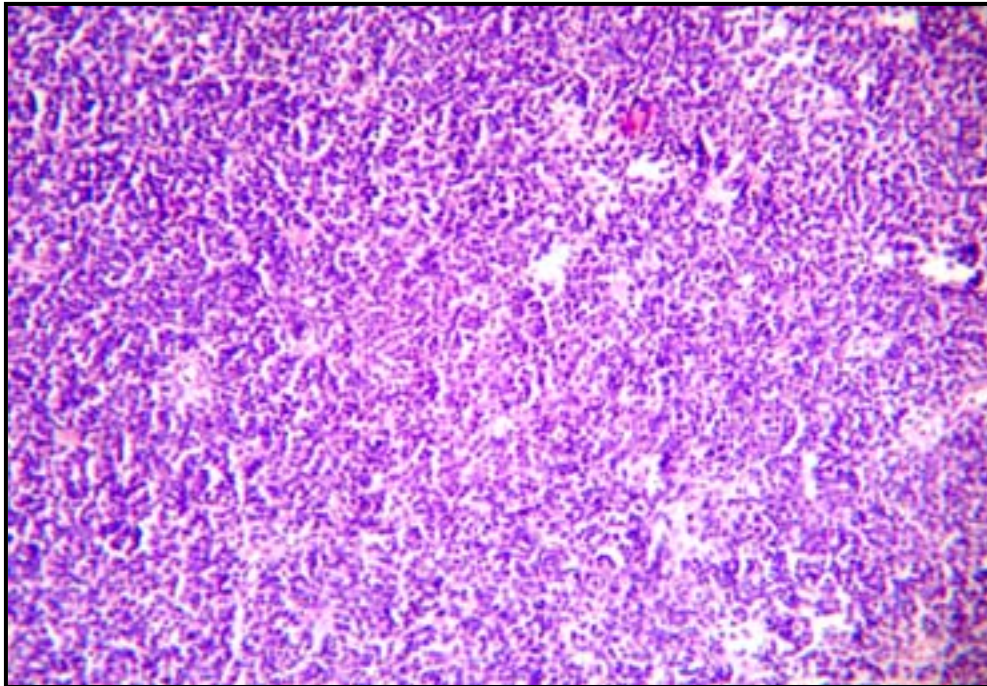


Fig 6: NHL Parotid gland: Shows diffuse arrangement of monotonous population of lymphoid cells H&E X 100(1546/10)

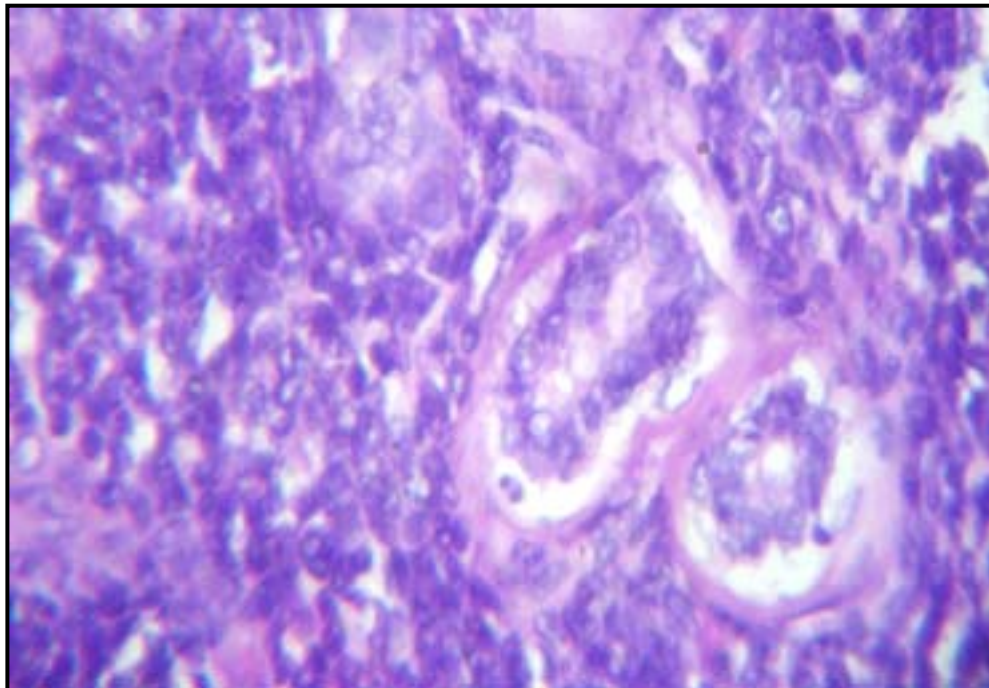


Fig 7: NHL Parotid gland: Shows salivary ducts surrounded by monotonous population of lymphoid cells H&E X 400(1546/10)

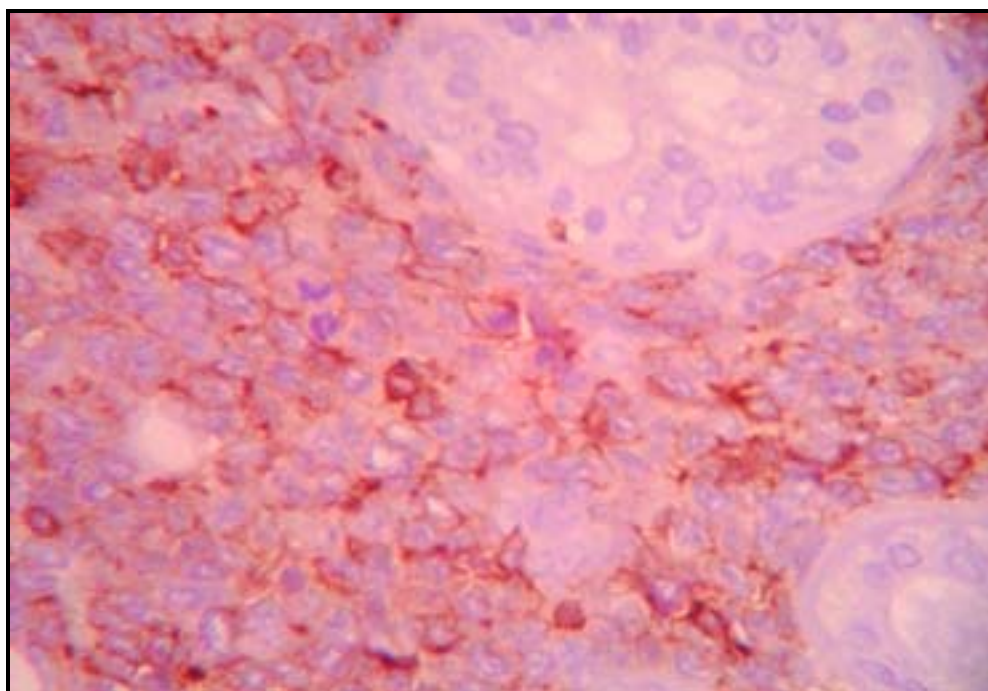


Fig 8: NHL Parotid gland: Lymphoid cells showing membranous positivity for CD 45 (LCA) IHC X400(1546/10)

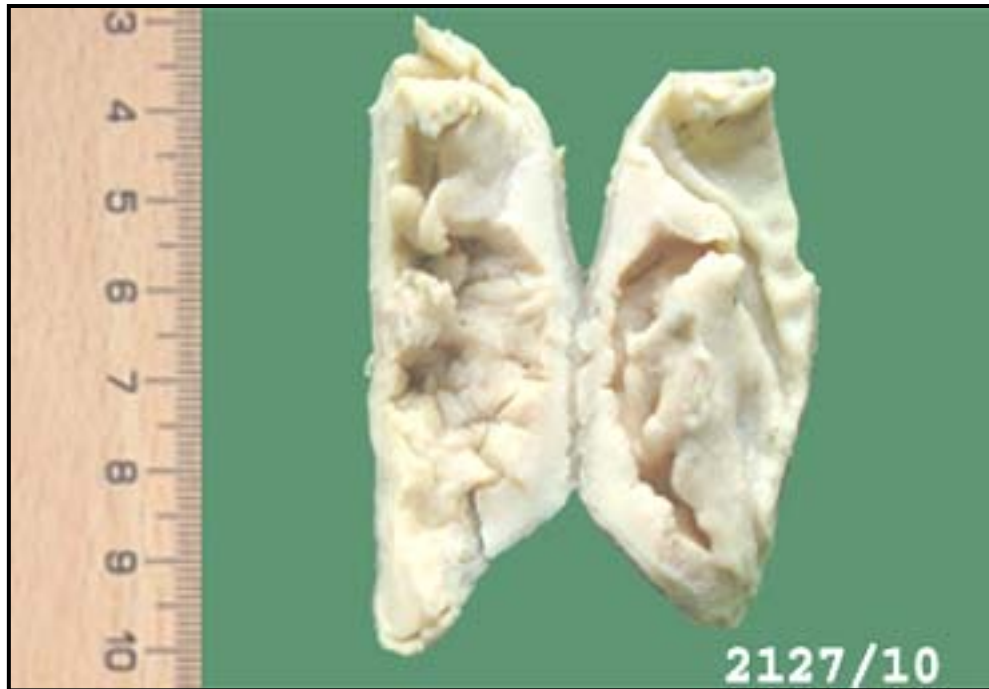


Fig. 9 :NHL Stomach: Cut surface shows homogenous gray white tumor involving more than 2/3 of stomach (2127/10)

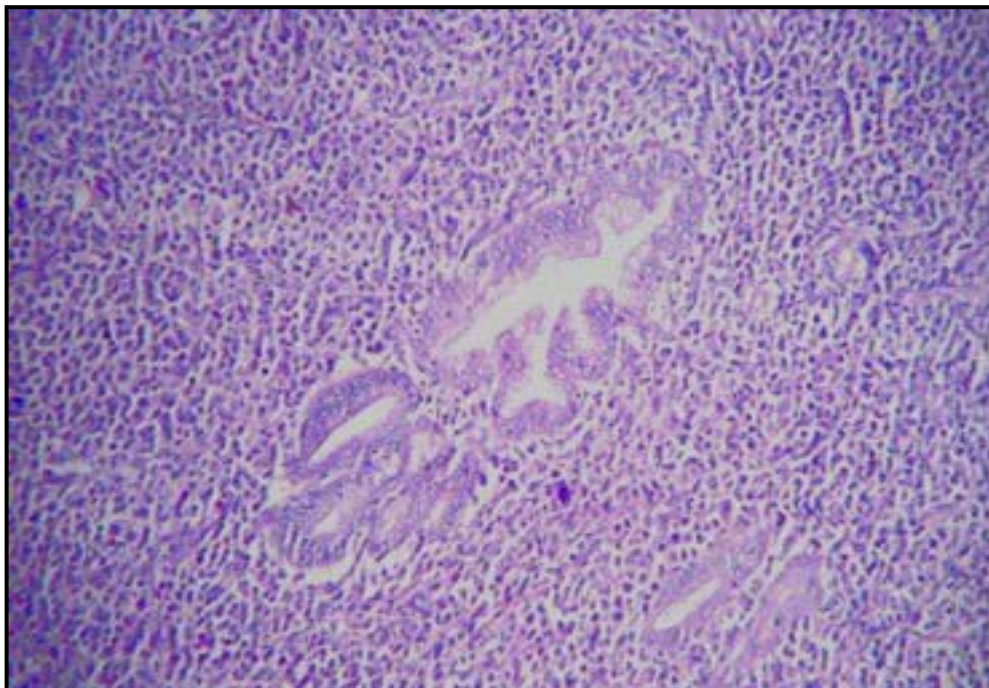


Fig. 10: NHL stomach: Shows mucosal glands surrounded by monotonous population of lymphoid cells H&E X100 (2127/10)

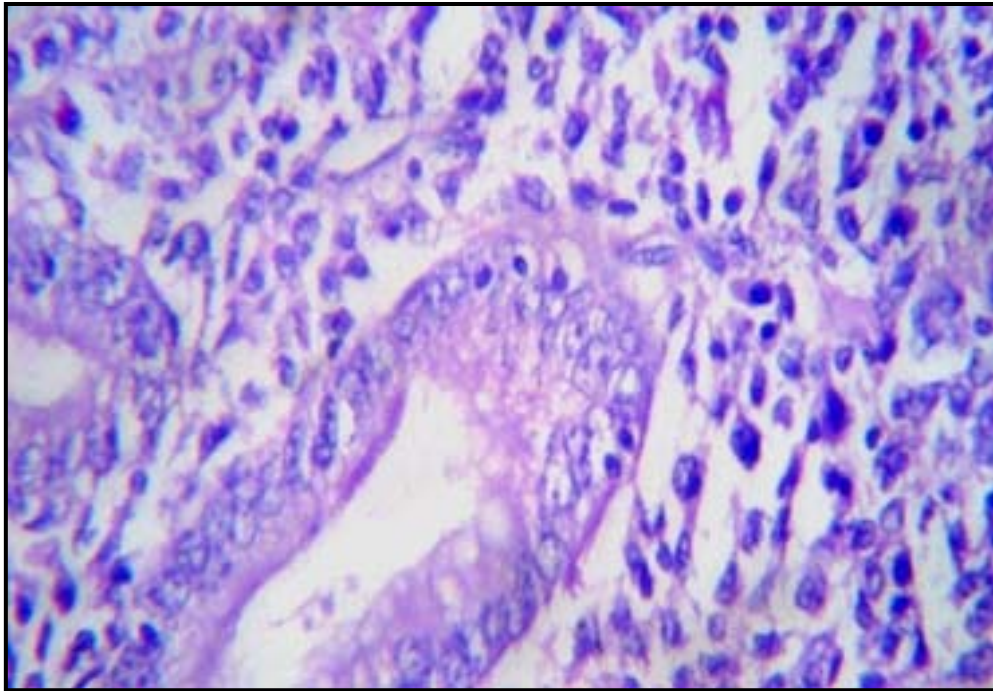


Fig 11:NHL stomach: Shows mucosal glands infiltrated by lymphocytes (Lymphoepithelial lesion) H&E X400(2127/10)

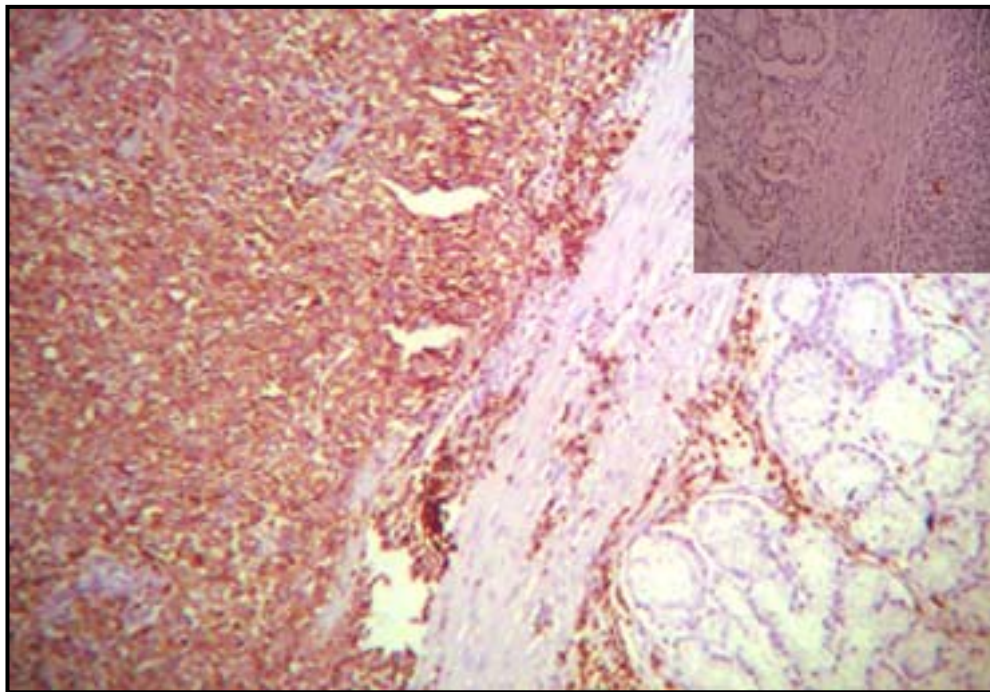
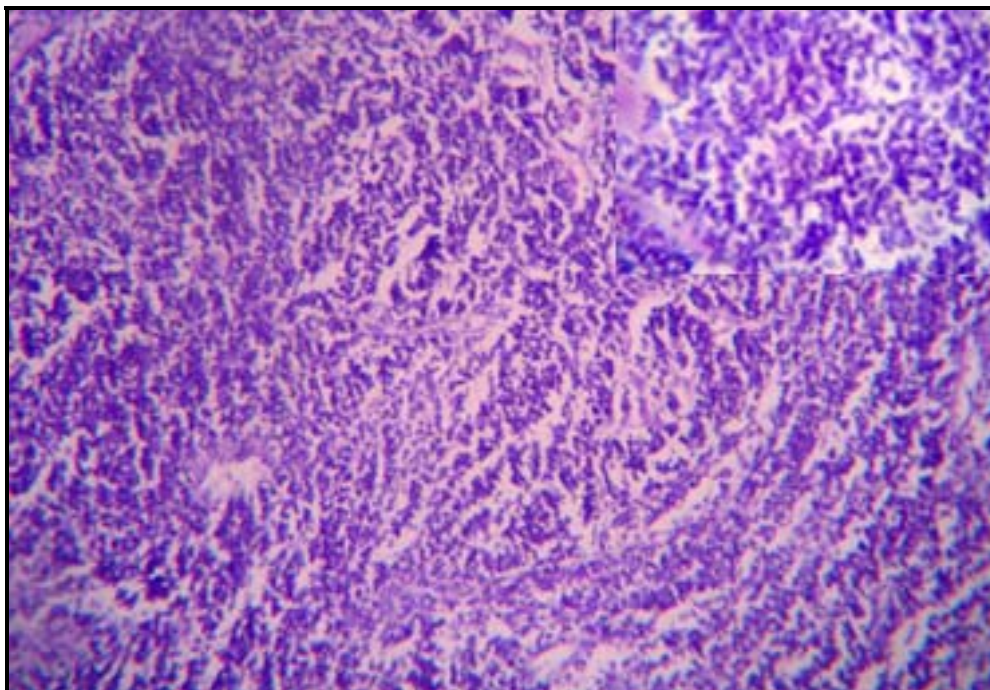


Fig 12 : NHL stomach: Tumor cells show membranous positivity for CD 45(LCA) inset shows negativity for cytokeratin X100 IHC (2127/10)



Fig 13: NHL small intestine: cut surface shows proliferative growth in distal end of ileum (2442/10)



**Fig 14: NHL small Intestine : monotonous proliferation of lymphocytes. H&E X 100
inset-Tumor cells with scanty cytoplasm and round nuclei H&E X 400 (2442/10)**

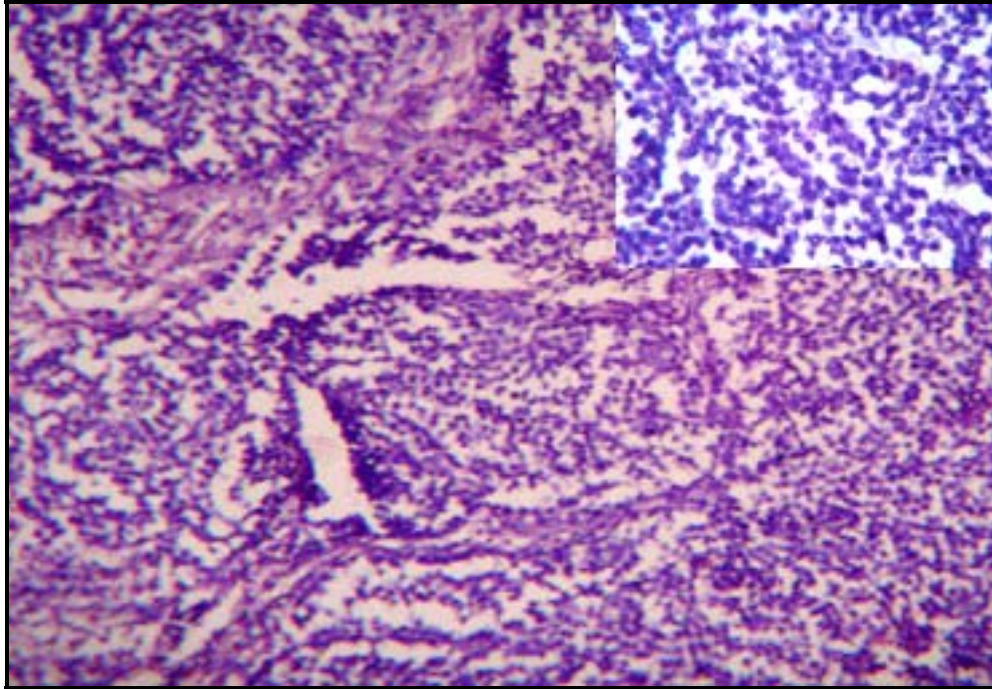


Fig15: Ewing's sarcoma/PNET : Uniform round blue cells arranged in lobules H&E X 100
Inset : Small round blue cells with rosette pattern H&E X 400 (3591/08)

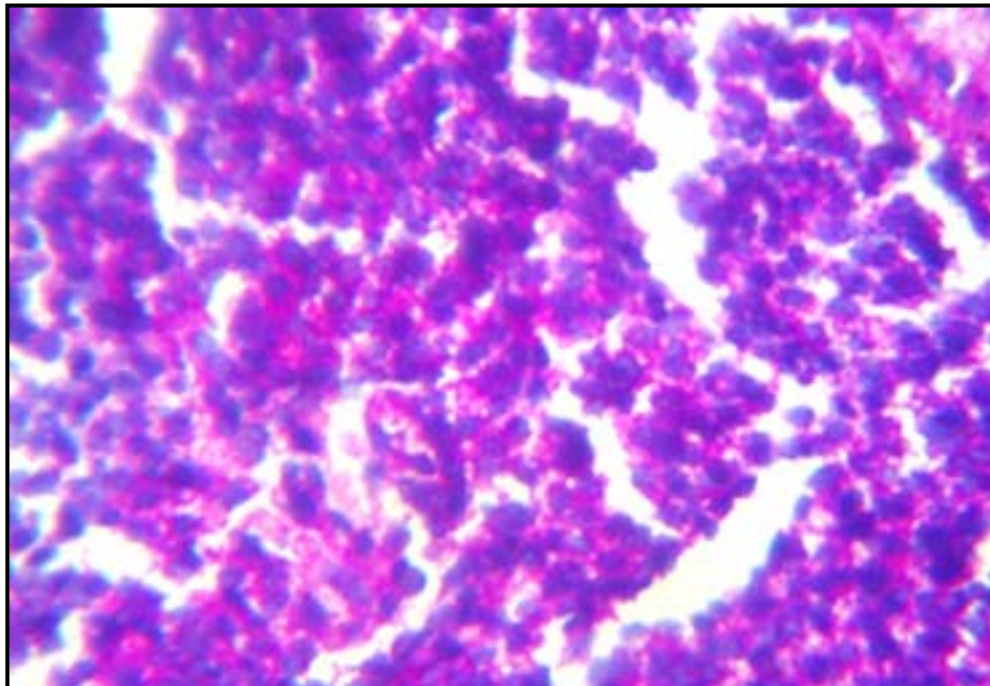
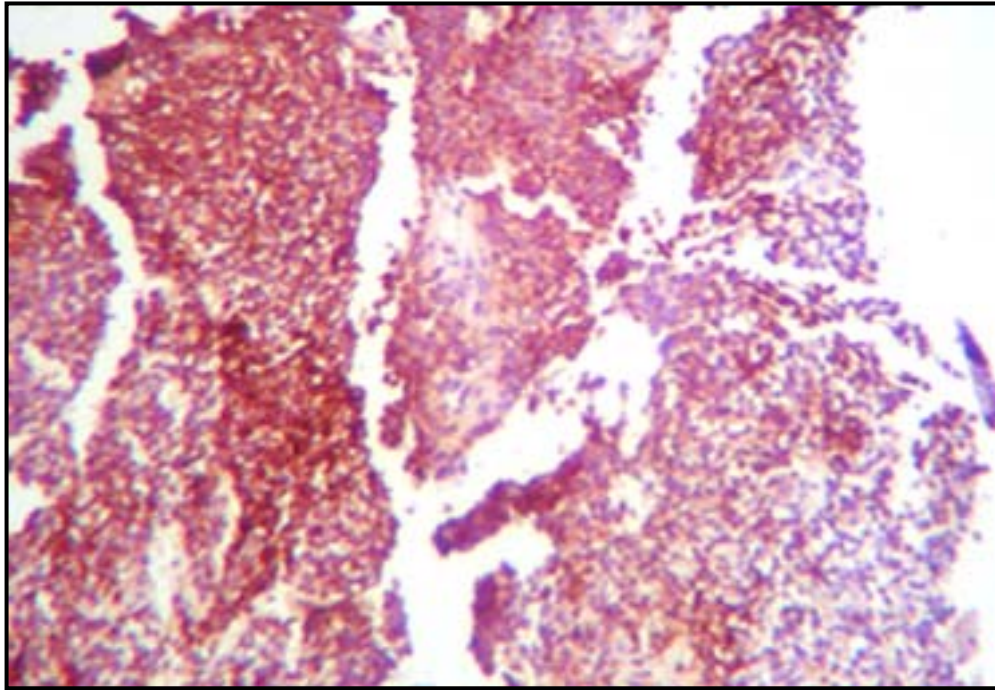
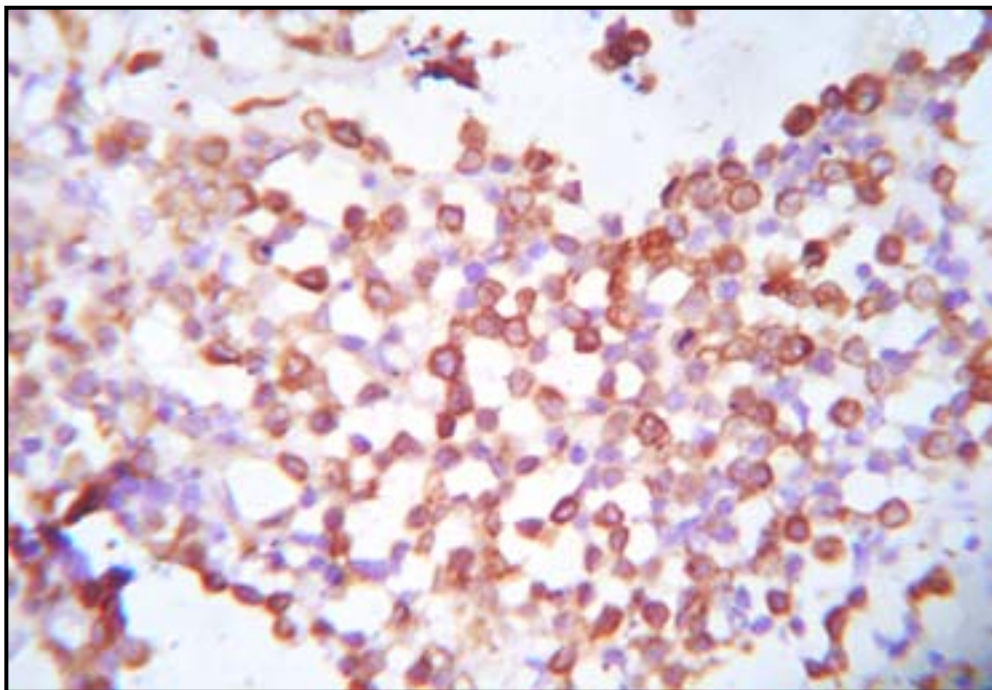


Fig 16: Ewing's sarcoma/PNET: Showing abundant cytoplasmic glycogen.PAS X400(3591/08)



**Fig 17: Ewing's sarcoma/PNET: Shows membrane immunoreactivity for CD 99
IHC X100(3591/08)**



**Fig 18: Ewing's sarcoma/PNET: Shows cytoplasmic positivity for vimentin
IHCX 400(3591/08)**

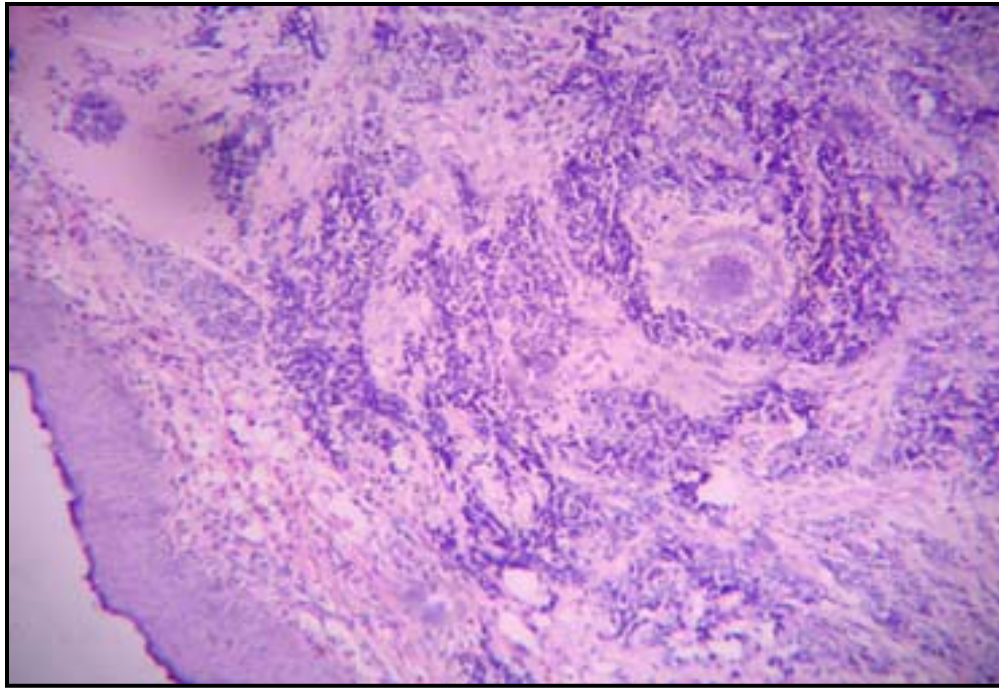


Fig 19: Rhabdomyosarcoma: Shows structure of skin with underlying small round blue cells arranged in nests and diffuse sheets H&EX100(3169/09)

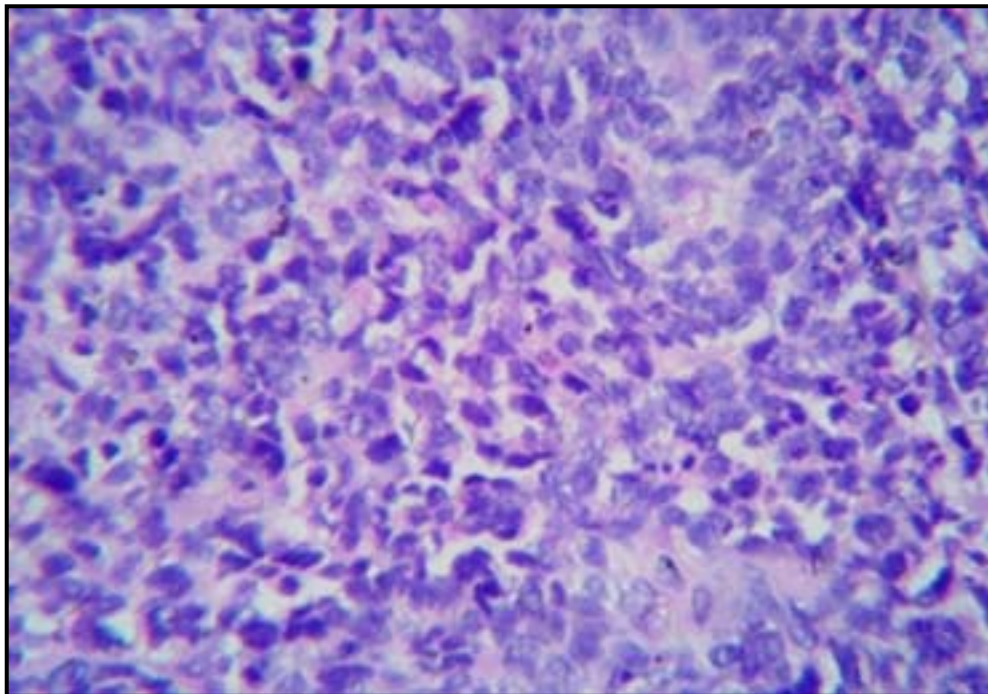


Fig 20: Rhabdomyosarcoma: Shows primitive round to ovoid cells with scanty cytoplasm and round blue nuclei H&E X400(3169/09)

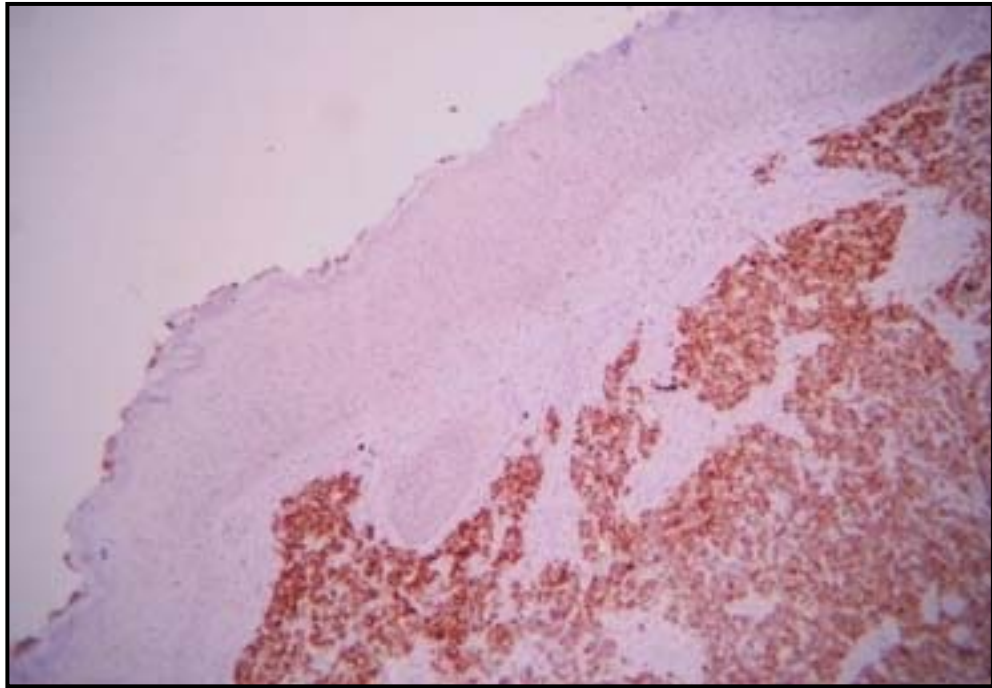


Fig 21: Rhabdomyosarcoma: Tumor cells show diffuse cytoplasmic positivity for desmin IHC X 100 (3169/09)



Fig 22: Alveolar RMS: Cross section shows grayish white to tan in colour (3593/09)

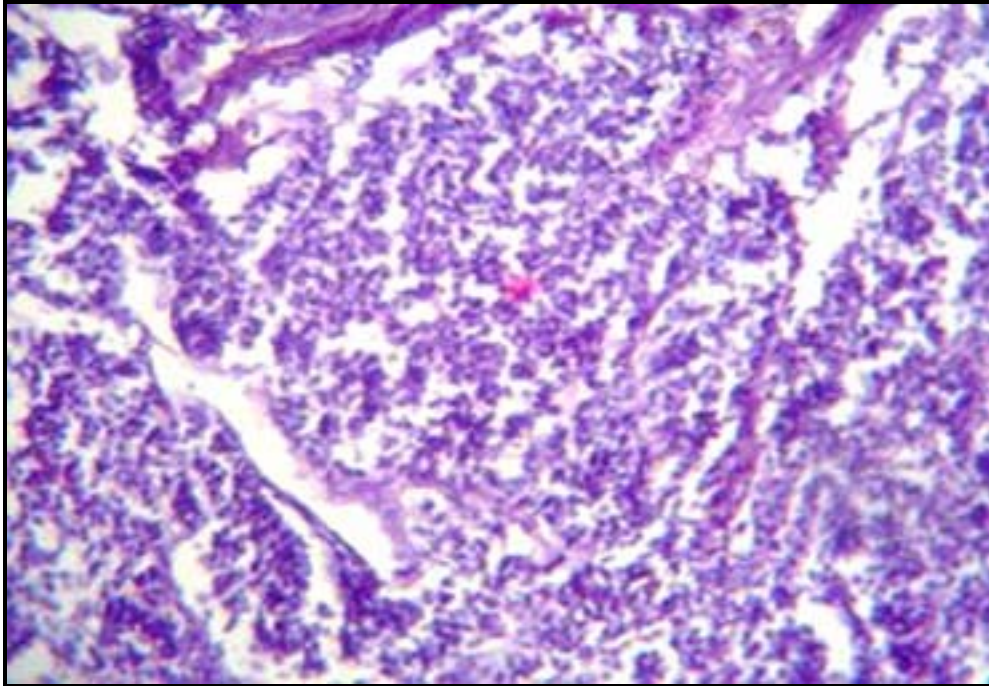


Fig 23: Alveolar RMS: Shows aggregates of tumor cells separated by thick fibrous septa with alveolar pattern H&E X100 (3593/09)

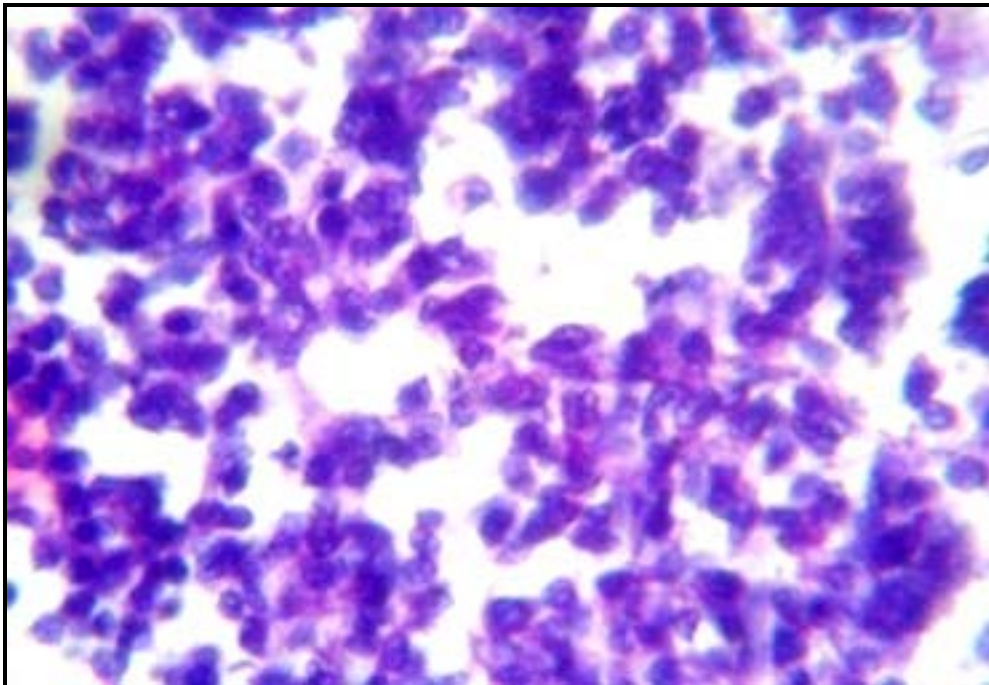


Fig 24: Alveolar RMS : shows round blue cells with scanty cytoplasm and round nuclei H&E X 400 (3593/09)



Fig 25: Wilm's tumor: Shows more than 2/3 of kidney replaced by gray white tumor tissue with hemorrhagic areas (2346/08)

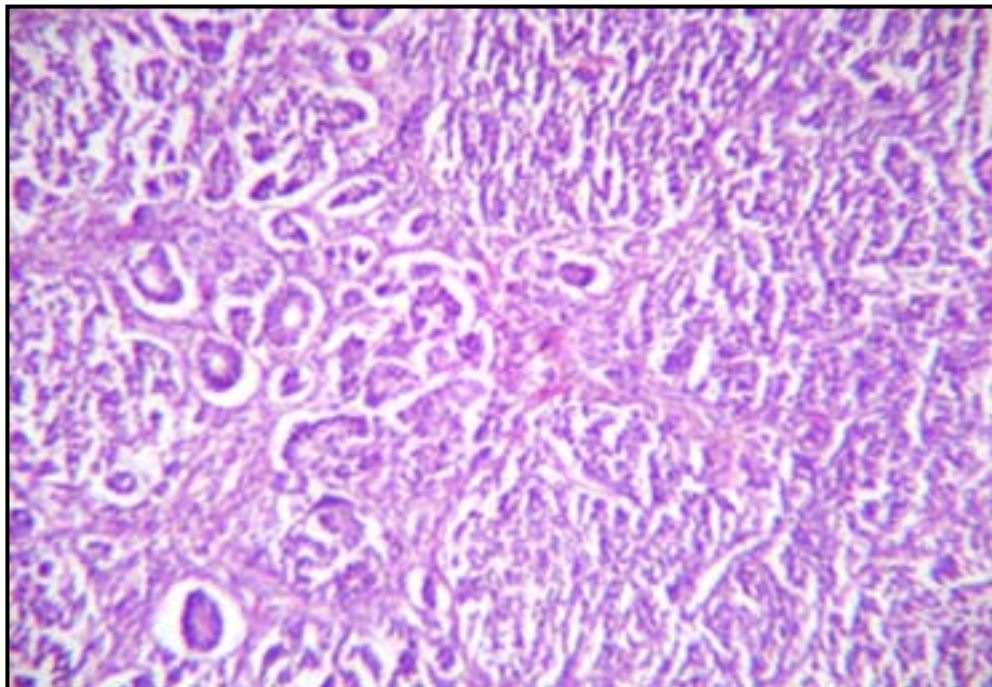


Fig 26: Wilm's tumor: shows combination of epithelial, blastemal and mesenchymal components H&E X100 (2346/08)

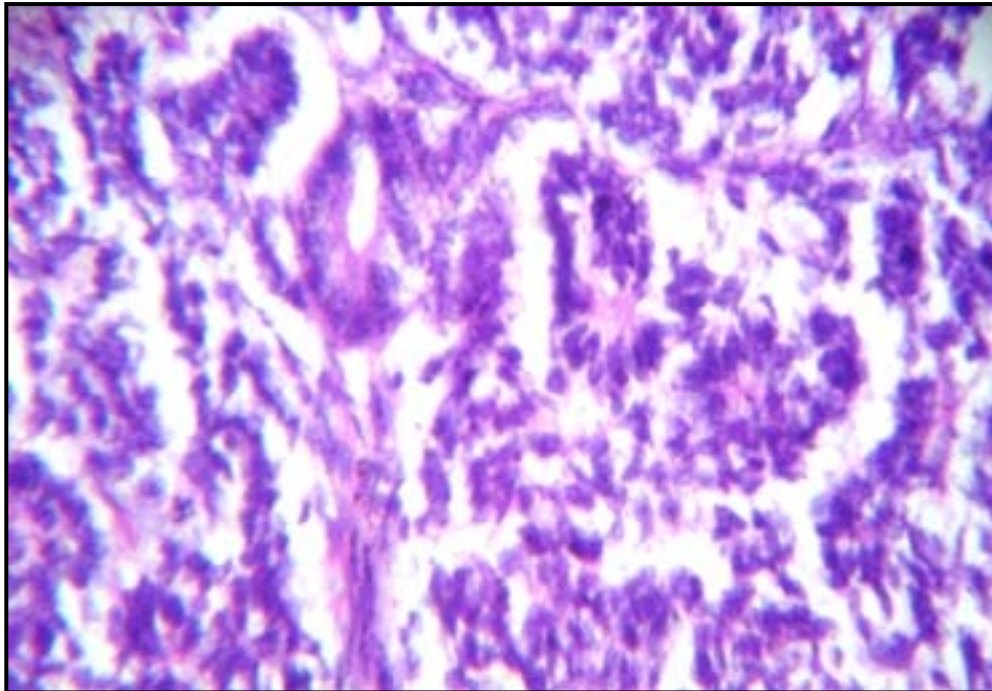


Fig27: Wilm's tumor: shows epithelial tubular formation, blastemal and mesenchymal components H&E X400 (2346/08)

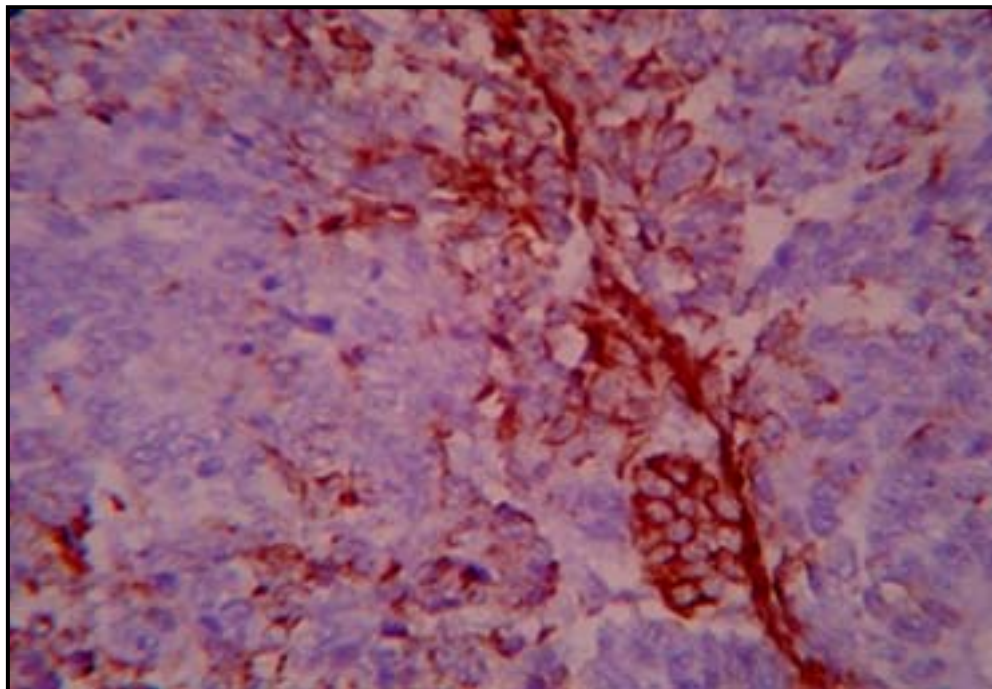


Fig28: Wilm's tumor: shows cytoplasmic immunoreactivity for vimentin IHC X400 (2346/08)

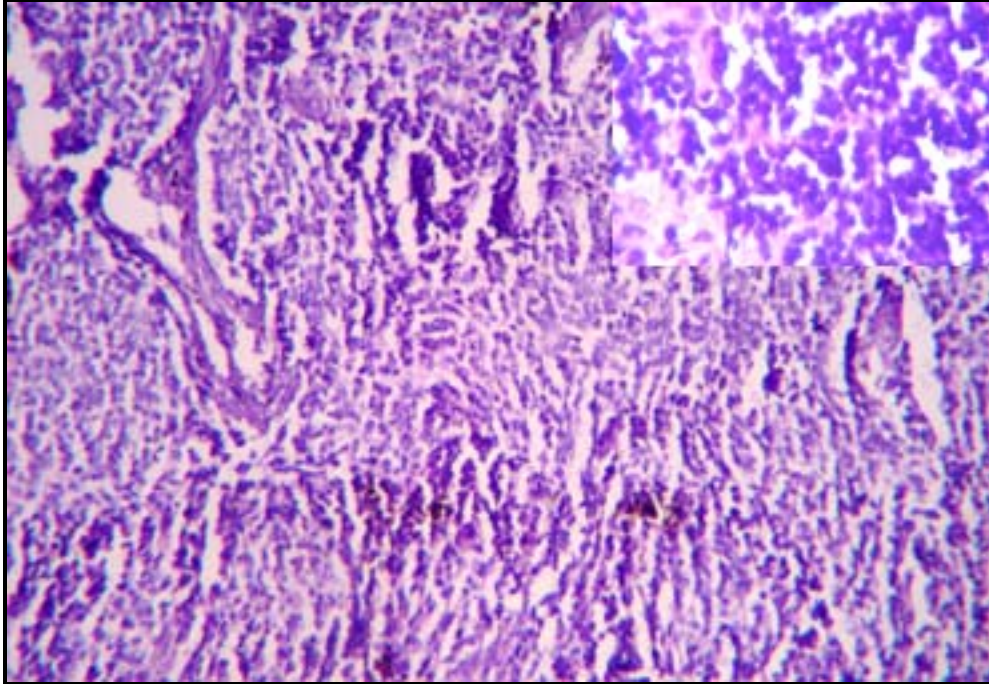


Fig 29: Neuroblastoma: Tumor cells arranged in nests and separated by delicate fibrous septa H&E X 100 Inset shows uniform round blue cells with rosette formation(1956/08)

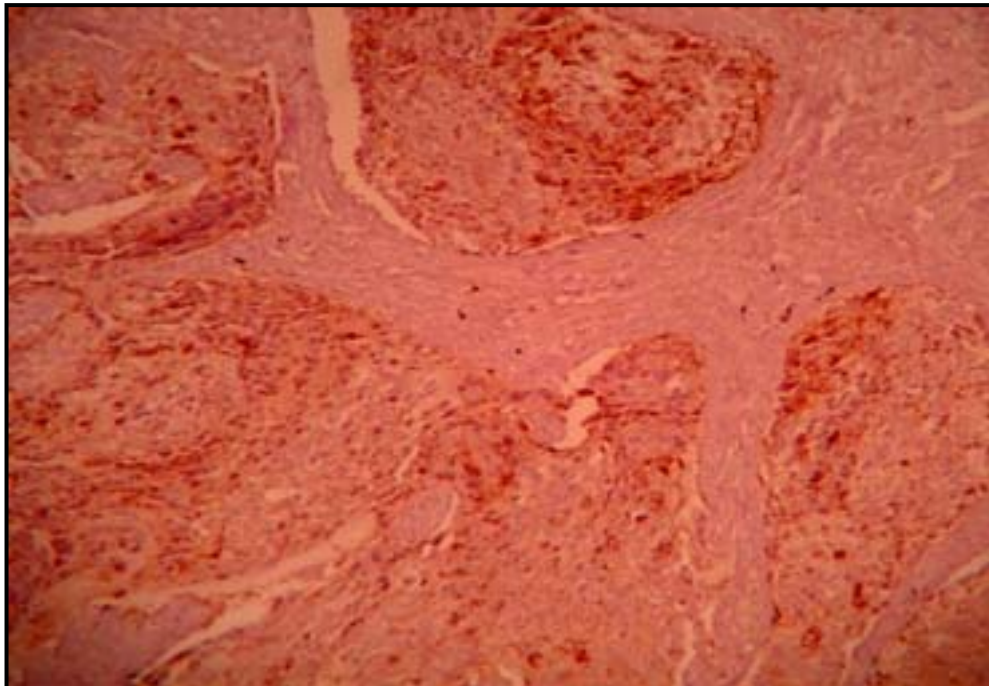
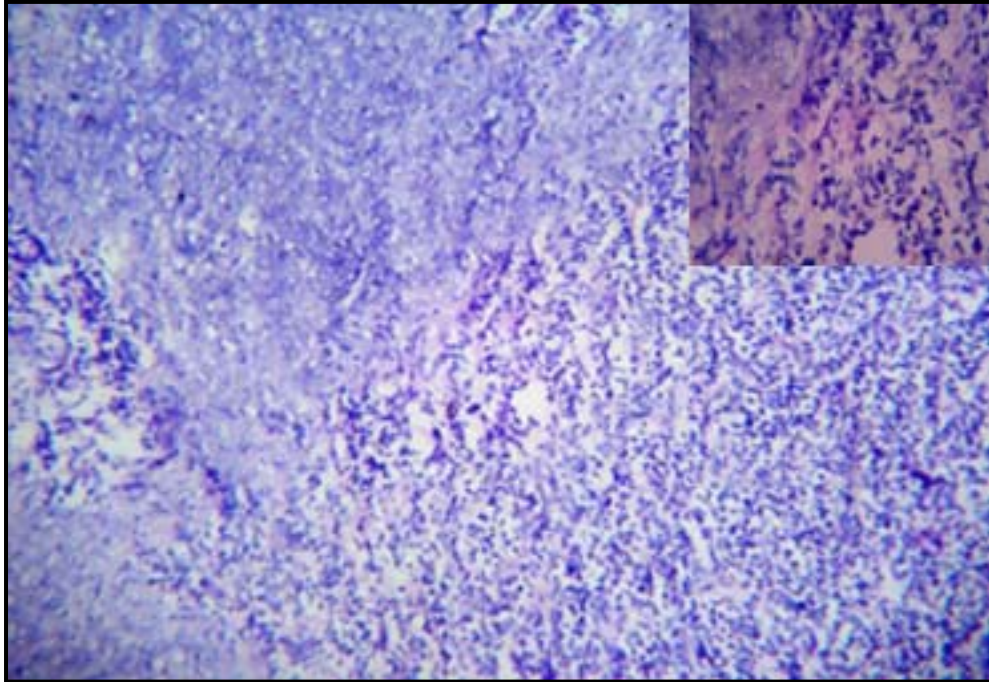


Fig 30: Neuroblastoma: Shows cytoplasmic granular positivity for chromogranin IHC X 100(1956/08)



**Fig 31: Extra skeletal Mesenchymal Chondrosarcoma: Shows bimorphic pattern with cartilaginous area and small round blue cells H & E X100.
Inset shows undifferentiated cells (3192/09)**

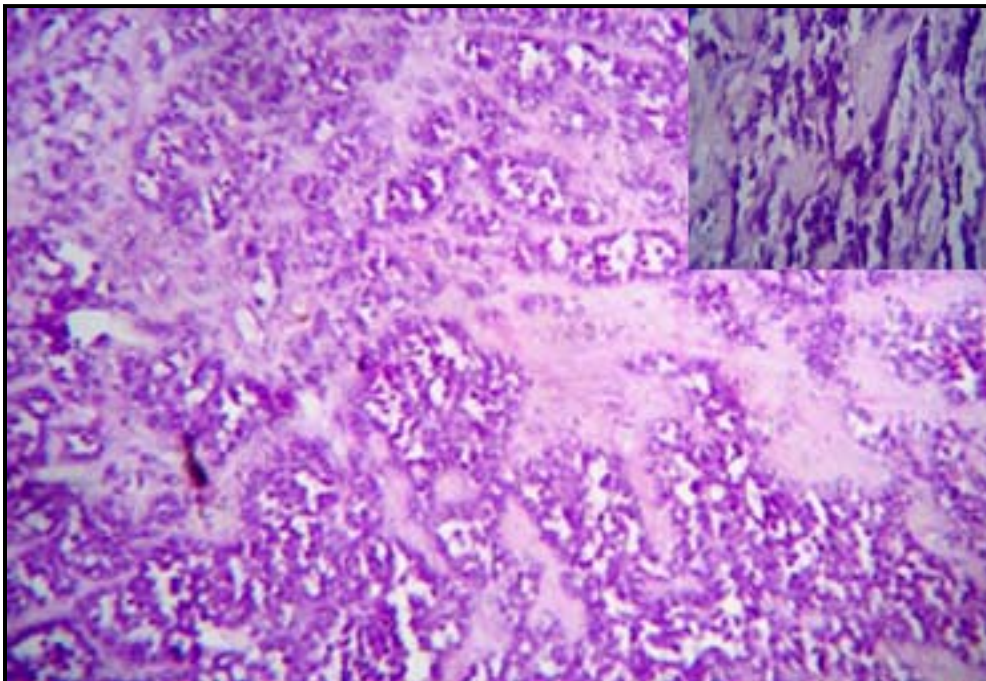
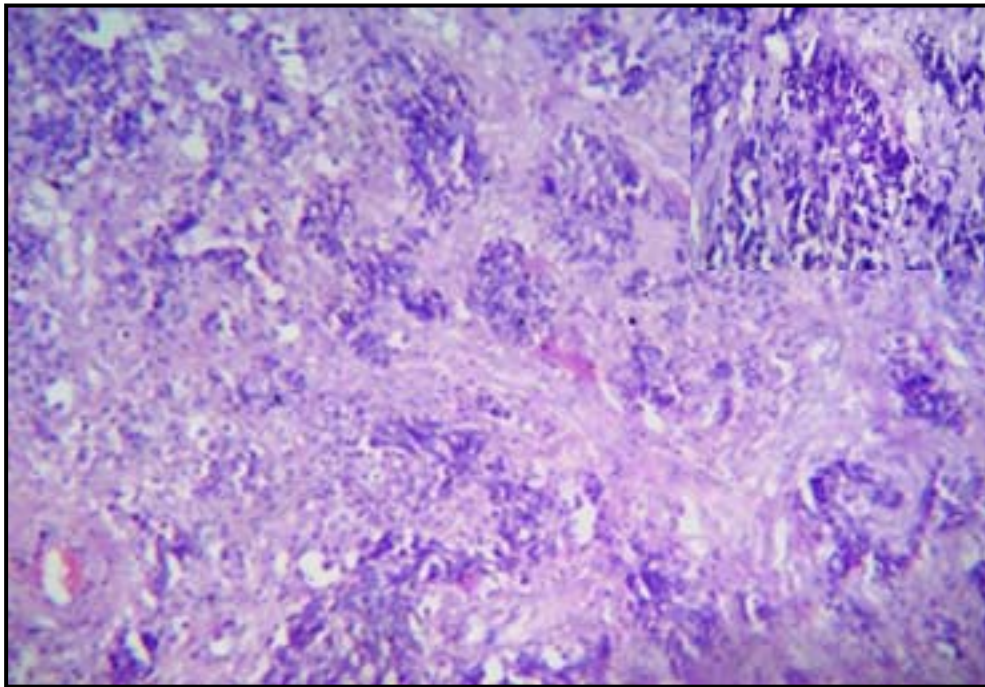
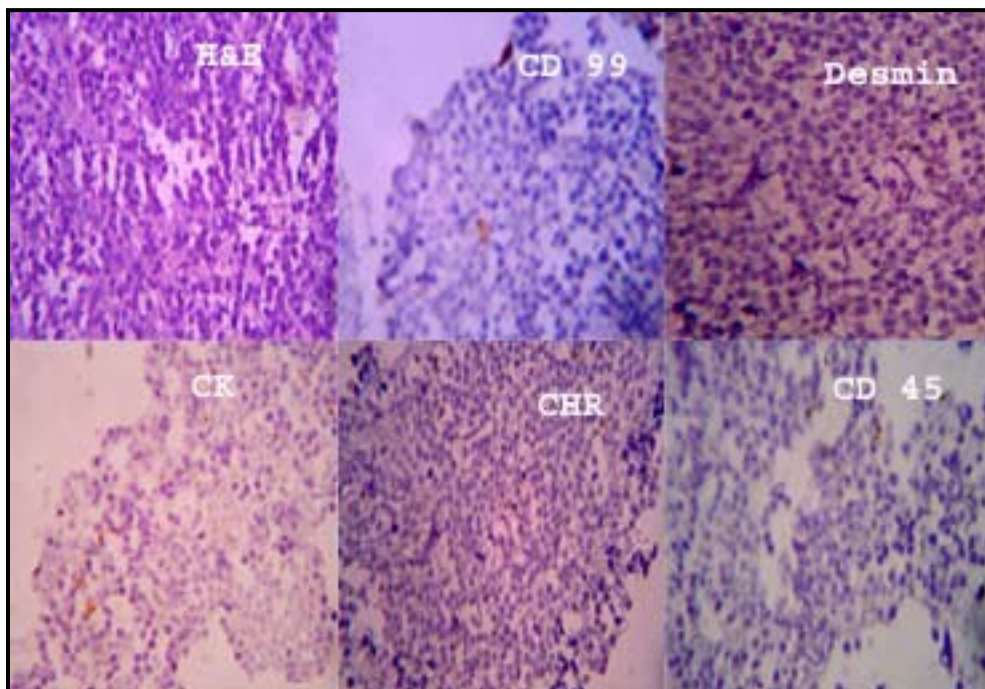


Fig 32: Small cell Osteosarcoma: Shows neoplastic osteoid with nests of tumor cells H&E X100.Inset shows small round cells (439/09)



**Fig 33: DSRCT: Shows nests small round cells surrounded by desmoplastic stroma
H&E X100. Inset shows nests of small round blue cells (3223/08)**



**Fig 34: Undifferentiated Malignant Round Blue Cell Tumor: Shows negativity in all of the
initial antibody markers IHC X 400 (1304/10)**